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CONTENTS OF VOLUME XXI.

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On the systematic relationships of the vinegar eelworm, *Turbatrix aceti*, and its congeners, with a description of a new species.

By T. Goodey, D.Sc.

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Study of a species of eelworm closely akin to the eelworm of paperhanger's paste but differing from it in certain anatomical details has afforded an opportunity for considering the systematic relationships of this interesting worm and its congeners with the result that a new and, to the writer, a more satisfactory grouping is put forward.

In the characters of the head, the structure of the stoma, the shape and build of the oesophagus and the general structure and arrangement of the female gonad as well as in the distribution of the male caudal papillae and the character of the spicules, the genera comprising this group show obvious resemblances to members of the family Cephalobidae Chitwood & Chitwood, 1934. The writer therefore proposes their formal inclusion in this family.

In having very fine transverse striations of the cuticle, in the general structure of the stoma, in the shape of the oesophagus (the corpus being without a distinctly swollen median bulb) with a narrow isthmus crossed almost medially by the nerve ring and a well developed terminal valvate bulb, the genera closely resemble those of the Panagrolaiminae Thorne, 1937. Likeness to the members of this sub-family is also shown in the build of the female gonad which is single and prodelphic; the ovary being reflexed and extending straight back well behind the level of the vulva without further flexures. The number and distribution of the male caudal papillae is also fairly similar to that found in males of that sub-family; some papillae being preanal, subventral and some postanal, subdorsal in position.

Differences from the subfamily Panagrolaiminae are shown in the rather large post-vulval uterine sac, in the stout muscular vagina and in the structure of the spicules which have bifid tips in some of the species. For these reasons, coupled with the fact of their ovoviviparous or viviparous habit, the writer deems it desirable not to include them within the Panagrolaiminae but to erect a new subfamily for their reception.

TURBATRICINAE NEW SUBFAMILY.

Diagnosis—Cephalobidae : Cuticular striations very fine. Head with blended or distinct lips. Stoma with cheilostom and protostom forming well defined chamber; cheilorhabdions conspicuous or inconspicuous, pro-, meso-, and metarhabdions and telorhabdions distinct or obscure. Oesophagus as in Panagrolaiminae; a fusiform muscular precorpus and corpus without distinct median bulb, isthmus crossed medially or posteriorly by nerve ring and terminal valvate bulb. Female gonad single, prodelphic, ovary reflexed straight back without further flexures and reaching in some cases to level of rectum. Distal end of uterus functioning as receptaculum seminis. Vagina non-muscular or muscular. A comparative large post-vulval uterine sac present. Male tail mostly with 7 pairs of caudal papillae, spicules sigmoid or ventrally arcuate.

Included in the new subfamily are 2 genera namely, *Turbatrix* Peters, 1927 and *Turbator* gen. nov.

TURBATRIX Peters, 1927.

Diagnosis : (emend). Turbatricinae. Body slender in both sexes. Tail long and sharply pointed in both sexes. Cuticle with very fine transverse striae. Lateral fields present, very narrow. Head formed from, probably, 6 thin incurved lips, overarching mouth aperture. 6 minute head papillae present in inner circlet round mouth. Stoma (fig. 1) about twice as long as wide made up of two principal parts; an anterior dome-shaped cheilostom with distinct cheilorhabdions and a posterior, funnel-shaped protostom with pro-, meso- and metarhabdions. Two subventral teeth present (possibly based on the metarhabdions) and a dorsal tooth more forwardly placed. Oesophagus typical. Vagina not specially muscular, ovary reflexed, post-vulval uterine sac fairly large. Male with sigmoid spicules, gubernaculum with a deep keel-like expansion (fig. 2).

Type species :—

Turbatrix aceti (Müller, 1783) v. *aceti* Peters, 1927. Vinegar eelworm.

One other form :—

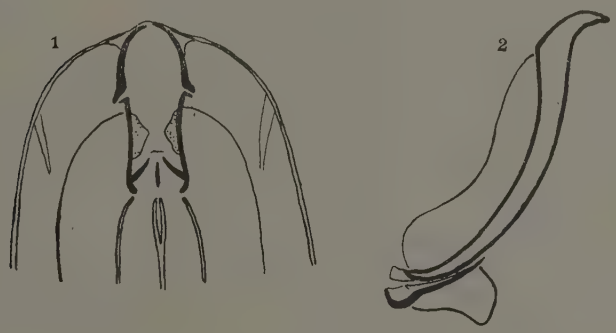
T. aceti (Müller, 1783) v. *dryophila* (Leuck. 1887) de Man, 1910. In white slime-flux of oak.

The other species which up to the present have been included in the genus *Turbatrix* show certain distinctive features in the structure of the

head, the shape and build of the stoma and the male spicules. The differences are sufficient to warrant the creation of a new genus for their reception, the proposed name for which is *Turbator*.

Turbator gen. nov.

Diagnosis : Turbatricinae. Head composed of 6 distinct conical or flatly rounded lips each with at least a small apical papilla (figs. 3–5). Stoma consisting of a cylindrical cheilostom, a little longer than wide, cheilorhabdions rather faint, and a short protostom with more prominent prorhabdions. Meso-, meta- and telorhabdions obscure but probably forming lining of initial funnel-shaped part of oesophagus. A minute



Turbatrix aceti v. *aceti*.

Fig. 1.—Head of female, dorsal view. ×2,400 (after de Man, 1910).

Fig. 2.—Spicule and gubernaculum, lateral view. ×1,300 (after de Man, 1910).

dorsal tooth present in one species (on mesorhabdion?). Oesophagus and intestine typical. Female gonad with muscular vagina and comparatively large post-vulval uterine sac. Uterus large with numerous eggs at a time. Spicules ventrally arcuate, more or less distinctly cephalated with head ventrally curved, tips bifid in 6 out of the 7 species, gubernaculum without keel-like expansion (Figs. 6–10).

Type species :—

Turbator redivivus (Linn, 1767). New comb.
syn. *Turbatrix rediviva* (Linn., 1767) Peters, 1927.
Anguillula rediviva (Linn., 1767) Stiles & Hassall, 1905.
Anguillula glutinis (Müller, 1783).
Cephalobus parasiticus Sandground, 1939.
The sour paste eelworm.

Other species :—

Turbator ludwigii (de Man, 1910).
syn. *Turbatrix ludwigii* (de Man, 1910) Peters, 1927.
Anguillula ludwigii de Man, 1910.
In white slime-flux of oak, Germany and England.

Turbator silusiae (de Man, 1913).
syn. *Anguillula silusiae* de Man, 1913.
In so-called beer felts, Germany and Alsace.

Turbator nepenthicola (Menzel, 1922).
syn. *Anguillula nepenthicola* Menzel, 1922.
In pitchers of pitcher plants, Dutch East Indies.

Turbator leucocephalus (Steiner, 1936).
syn. *Neocephalobus leucocephalus* Steiner, 1936.
In an agar culture inoculated with wood from scarlet oak and apparently feeding on a fungus, U.S.A.

Turbator pycnus (Thorne, 1938).
syn. *Panagrellus pycnus* Thorne, 1938.
In a slime-flux from a Great Plains cottonwood tree, *Populus sargentii* Dode, near Magna, Utah, U.S.A.
Turbator ridivivoides n. sp.

In the above list it will be noted that in addition to the species previously included in the old genus *Anguillula* and now referred to *Turbator,* the writer has formally transferred the two species *Neocephalobus leucocephalus* and *Panagrellus pycnus* to the newly erected genus *Turbator.* This has been down on the following morphological grounds. In both species the adults of both sexes have long pointed tails. In the characters of the head, the stoma, the oesophagus they resemble *Turbator* closely. As also in having a fairly long muscular vagina, directed inwards and forwards, and a large roomy uterus. *Neocephalobus leucocephalus* possesses a large post-vulval uterine sac but in *Panagrellus pycnus* it is not clear whether such a sac is present.

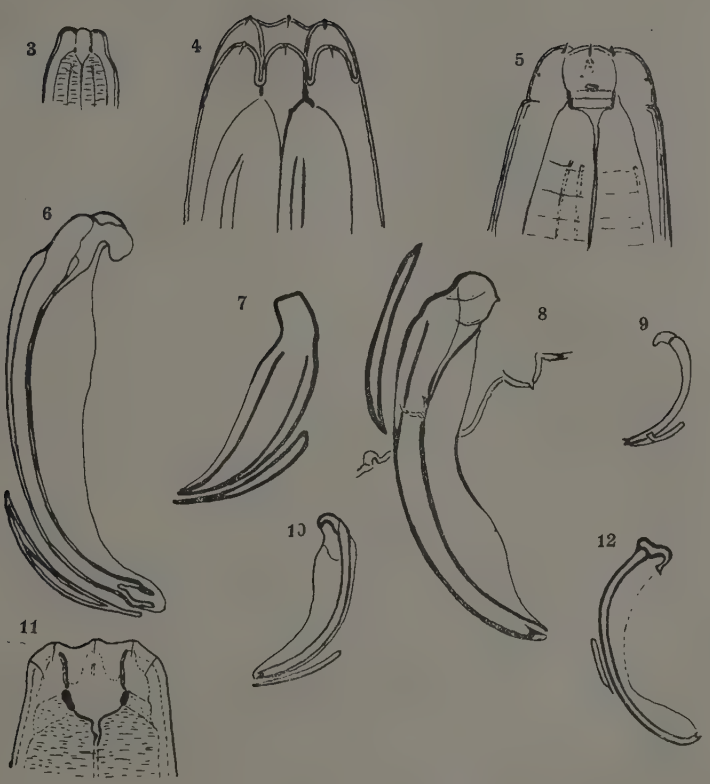


Fig. 3.—Head of *Turbator redivivus*, lat. view. × 450 (after Goodey, 1922).

Fig. 4.—Head of *Turbator ludwigii*, lat. view. × 1,500 (after de Man, 1910).

Fig. 5.—Head of *Turbator leucocephalus*, lat. view. ×1,400 (after Steiner, 1936).

Fig. 6.—Spicule and gubernaculum of *T. redivivus* lat. view. ×1,040 (after Goodey, 1922).

Fig. 7.—Spic. & gub. of *T. ludwigii*, lat. view. ×1,500 (after de Man, 1910).

Fig. 8.—Spic. & gub. of *T. nepenthicola*, lat. view. ×890 (after Micol. & Menzel).

Fig. 9.—Spic. & gub. of *T. leucocephalus*, lat. view. ×530 (after Steiner, 1936).

Fig. 10.—Spic. & gub. of *T. silusiae*, lat. view. ×600 (after de Man, 1914).

Fig. 11.—Head of *T. pycnus*, lat. view. ×1,500 (after Thorne, 1938).

Fig. 12.—Spic. & gub. of *T. pycnus*, lat. view. ×500 (after Thorne, 1938).

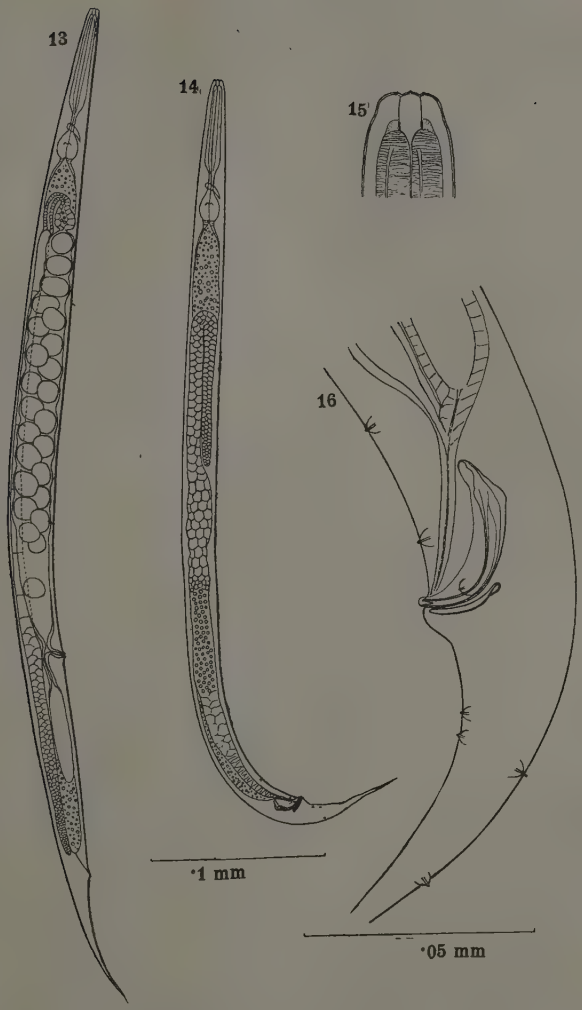
One is not figured but it would be of great interest to know if one is present. Possibly it was overlooked. The spicules of *N. leucocephalus* and *P. pycnus* are ventrally arcuate, have bifid tips and are cephalated with a forward hook on the head. In *P. pycnus* also there is a ventral membrane extending from the tip to the head. The distribution of the male caudal papillae in these two species is on the same general plan as that of other species of *Turbator;* particularly one may note the presence of the postanal subventral pair. Added to these structural features is the fact of the natural habitat of the worms. Both species were found associated with trees and *P. pycnus* was obtained from the slime-flux of a cottonwood tree; a most significant fact. Should subsequent observations show that the female of *P. pycnus* does not possess a post-vulval uterine sac this would be a character differentiating it from the other species of the genus *Turbator* and it might then be advisable to retain *Panagrellus* as a distinct genus and instead of the subfamily Turbatricinae containing the two genera *Turbatrix* and *Turbator* only, *Panagrellus* would also be included.

Turbator rediviroides n. sp.

Morphology.

Dimensions: *Female,* length, 0·98 mm. to 1·7 mm. $a=17$–24, $\beta=5$–8, $\gamma=8$–10, V = 64%–70%. *Male,* length, 0·81 mm. to 1·28 mm. $a=23$–27, $\beta=5$–$6{\cdot}5$, $\gamma=8$–10, spicules 37μ–45μ, gubernaculum, 14–18μ.

Adults very similar in appearance and structure to those of the sour-paste eelworm, *Turbator redivivus*. Body tapering slightly in front to the blunt end and considerably behind to the long sharp tail. Cuticle with numerous delicate longitudinal striations each carrying very fine transverse striae. Head slightly offset, composed of 6 rather flatly rounded lips, each bearing a small apical papilla; the 6 being closely set round the mouth aperture. Other head papillae not seen. Stoma about one and a half times as long as wide and one-third the width of the head. Made up of a cheilostom with faint cheilorhabdions and a short protostom, embraced by a brief extension of the oesophagus, the walls consisting of short, rod-like prorhabdions. Distinct meso-, meta- and telorhabdions indistinguishable as separate elements, but probably forming the slightly thicker lining of the funnel-shaped beginning of the oesophagus lumen. Oesophagus with stout, muscular procorpus blending with corpus to form first part; a distinct median bulb being absent. Isthmus rather short and expanding into the rather large valvate terminal bulb. Nerve ring crossing isthmus, excretory



Turbator redivivoides n. sp.

Figs. 13 & 14.—Adult female and male respectively, lat. view showing general shape and structure.

Fig. 15.—Head highly magnified showing papillate head and structure of stoma.

Fig. 16.—Male tail, lat. view, highly magnified, showing distribution of caudal papillae and shape of a spicule and gubernaculum.

pore obscure but ventrad to nerve ring. Intestine richly stocked with fat globules.

Female. Vagina muscular. Gonad single, prodelphic, reflexed, ovary extending straight back with its tip at about level of end of intestine. Uterus roomy and containing a large number of eggs at a time. Sperms stored at anterior end of uterus. Post-vulval uterine sac comparatively large and reaching in some specimens more than halfway from vulva to anus. Ovoviviparous or viviparous.

Male. Tail always somewhat ventrally bent and sharply pointed. Gonad single, anteriorly reflexed for some distance. Vas deferens narrowing posteriorly to form ejaculatory duct which opens into beginning of rectum to form the cloaca. 7 pairs of caudal papillae present situated as shown in fig. 16. 1 and 2 preanal, subventral; 3 adanal, almost lateral and rather inconspicuous; 4 and 5 forming a postanal, subventral pair fairly close together; 6 and 7 postanal and subdorsal. In *T. redivivus*, the writer (1922) figured only 5 pairs of caudal papillae but it seems quite possible that those corresponding to Nos. 3 and 6 may have been overlooked. Spicules paired, shaped as shown in fig. 16, when seen in lateral aspect. If this is compared with fig. 6, a spicule from *T. redivivus*, the difference in shape is at once apparent. In the new species the head end is somewhat drawn out and tapers to a rather small forward hook. It is not, in fact, so definitely cephalated as in *T. redivivus*. The shaft from the broadest region tapers backwards over a considerable distance to the bifid tip whereas in *T. redivivus* the shaft has about the same width throughout most of its length and then expands to form the head. As in *T. redivivus*, a membranous expansion extends from the head to the tip on the ventral side and surrounds the tip. The gubernaculum also has a different shape from that of *T. redivivus* and constantly shows the distal end folded over to form a kind of loop. It is mainly on the different shape of the spicules and the gubernaculum that the writer has separated the new species from *T. redivivus*.

Occurrence.

The worms were found originally in a banana maize-meal cider culture which had been exposed at Cambridge in order to attract *Drosophila* flies. About three weeks after being visited by the flies, two cultures were found to be populated with large numbers of eelworms and shortly afterwards a portion of the cultures was sent to the writer with a request for the identification of the worms. There can be no

doubt that the inoculum of eelworms was originally brought to the culture on the bodies of the visiting flies; a similar association between *Drosophila* flies and *T. silusiae* having been demonstrated by Aubertot (1925) in Alsace. The worms from Cambridge were successfully sub-cultured for some months on a flour paste medium and were, in fact, taken to be the sour-paste eelworm. It was only when they came to be examined in detail that the differences noted above were constantly found.

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Journal of Helminthology, Vol. XXI, *No.* 1, 1943, *pp.* 10–17.

On *Rhabditis curvicaudata* (Schneider) and *R. paraciliata* n. sp.

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Schneider (1866), under the name *Leptodera curvicaudata*, described and figured certain nematodes obtained from moist soil and decaying matter. The species was removed to the genus *Rhabditis* by Oerley (1886) and the name became *R. curvicaudata* (Schneider, 1866) Oerley, 1886. Apart from the original description there appears to be no further record of the species in the literature dealing with this genus of free-living nematodes. The discovery of adult worms which agree well with Schneider's account provides an opportunity for redescribing the species and for giving some account of its very interesting association with flies belonging to the genus *Psychoda*. The writer is indebted for the material to Mr. G. H. Satchell who is investigating sewage purification problems at the University of Leeds (under a grant made by the Department of Scientific and Industrial Research) and discovered the association between the larvae of the worms and *Psychoda* flies concerning which further particulars are given later on.

The second species dealt with is another species of *Rhabditis* examples of which were sent to the writer for identification in 1940 by the Imperial Institute of Entomology. They had come originally from a rotting oil palm in Malay. Study of these worms has shown that they are very similar to *Rhabditis ciliata* Fuchs, 1931, from which, however, they differ in certain anatomical features and a new species is made for their reception.

1. *Rhabditis curvicaudata* (Schneider, 1866) Oerley, 1886.

Morphology.

Dimensions: *Female*, length, 1·12 mm. to 1·47 mm., $a=15$–20, $\beta=4{\cdot}4$–5, $\gamma=13$–17, V = 55%–61%; average of 12 specimens, length, 1·23 mm., $a=17$, $\beta=4{\cdot}5$, $\gamma=16$, V = 57%. *Male*, length, 0·99 mm. to 1·27 mm., $a=17{\cdot}5$–21, $\beta=4{\cdot}2$–5, $\gamma=11{\cdot}7$–$15{\cdot}5$, average of 7 specimens, length 1·12 mm., $a=19{\cdot}2$, $\beta=4{\cdot}6$, $\gamma=13{\cdot}4$.

The body in both sexes is moderately stout and tapers slightly anteriorly in the oesophageal region to the head end. It does not taper much posteriorly in either sex but ends in both male and female in a

fine caudal process which is terminally situated and is generally curved a little to one side and it is on this character that Schneider based his specific name.

The cuticle appears to lack transverse striations. The head is offset by a distict constriction. It is made up of six lips each of which is roundly conical in shape and appears to be provided with 3 small papillae; one apical and two somewhat laterally directed. The stoma consists of a straight protostom about 18-20μ long by about 7μ wide. Posteriorly it leads into the telostom the more delicate walls of which end in small knob-like telorhabdions. The oesophagus consists of an anterior almost cylindrical corpus which leads to the gently swollen median bulb. The latter gradually tapers into the isthmus, which is crossed by the nerve ring almost medially and then expands into the rather large terminal bulb containing a valve apparatus. The excretory pore is difficult to find but is located a little anterior to the level of the terminal oesophageal bulb.

Female. The anus is situated close to the rounded end of the body. At the base of the terminal caudal process there appears to be a small papilla on each side of the body, though it is not constantly present. The gonads are paired, opposed and reflexed, the terminus of the ovary reaching, in some cases, almost to the level of the vulva. The latter is a little post-equatorial in position. The short vagina leads inwards at right angles to the ventral surface and opens into the common tube formed by the junction of the two uteri. Each uterus is comparatively short and usually contains from 1–3 eggs at a time. The distal end is a little swollen and serves as a receptaculum seminis. It leads into a distinct cellular oviduct the proximal end of which has the structure of a sphincter (fig. 3). The oviduct opens into a thin walled tube containing developing eggs of gradually diminishing size which is finally reflexed and is continued into the ovary.

Male. The male tail tapers gently from a short distance anterior to the heads of the spicules and then more steeply in the postanal region. The cuticle of the tail is inflated to form a bursa. The latter is supported by 10 pairs of caudal papillae or rays which are arranged as shown in figures 4 and 5. 1 is preanally situated and is directed rather laterally, 2 and 3 form a pair practically adanal in position, 4 is directed ventro-laterally and is immediately followed by 5, 6 and 7, a somewhat greater distance separating 5 and 6 than 6 and 7; 8 is somewhat postero-laterally directed whilst 9 and 10 form a pair ventrally directed.

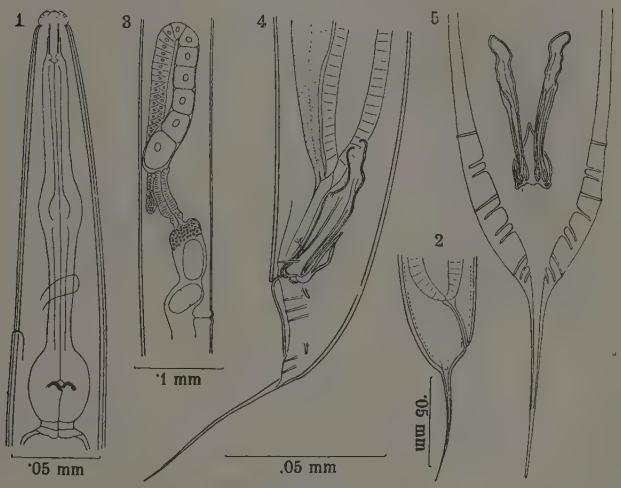
The paired spicules are rather large and are about 50μ long. They are shaped as shown in figures 4 and 5. The head end of each is rather drawn out and forms a kind of crooked handle with the shaft of the spicule forming a kind of broad knife blade. Each spicule tapers slightly towards the tip which is broad and rounded with a median indentation when seen in lateral aspect. A ventral view of the spicules shows that the tips are rather broad and each appears to consist of two bifid branches.

The gubernaculum is about one-third the length of the spicules. It is shovel-shaped with the broadest part underlying the tips of the spicules and also curving a little round their sides. The gonad is single, reaches well forward in the body and the anterior end of the testis is reflexed for a short distance. The vas deferens narrows down to form an ejaculatory duct which joins the ventral wall of the rectum quite close to the end of the intestine so that a common cloacal duct is formed which lies in between the two spicules and leads direct to the cloacal aperture. Special ejaculatory glands appear to be lacking. The shape of the female and the male tail, the arrangement of the bursal papillae and the general shape of the spicules agree well with these features as represented in Schneider's very small drawings of *R. curvicaudata* and consequently the specimens studied by the writer have been placed in this species.

Bionomics.

As already mentioned, larvae of the nematode were found by Mr. G. H. Satchell to have an intimate association with *Psychoda* flies breeding in certain sewage filter beds in the vicinity of Leeds. He also found that when cultures were made of steamed *Phormidium* growth scraped from the surface of the beds and then exposed to various captured *Psychoda* flies, the cultures afterwards became heavily infested with nematodes. Some of the larvae from such a culture and one from a captured fly were sent to the writer for identification and it was found that they belonged to the genus *Rhabditis*. In reporting on these worms the writer directed attention to the work of Bovien (1937) who had found a rather remarkable association between the larvae of certain species of *Rhabditis* which were attached to the surface of *Psychoda* flies present in large numbers in a jar of cow dung. Bovién at first regarded these nematode larvae as endoparasites of the flies, but on closer examination found that they were " attached to the exoskeleton in a peculiar manner." He further says: " If the fly is examined with

fairly high magnification, you may find the worms in the intersegmental furrows twined round the abdomen in such a way that they have the appearance of tightly fitting rings. As they are more or less concealed by the rich hair-covering of the fly, they may easily be overlooked or taken for endoparasites, when the abdomen is torn with needles. If, however, the flies are poured over with hot alcohol, the nematodes will straighten themselves and sink to the bottom. In this way I learned the true connection."



Rhabditis curvicaudata.

Fig. 1.—Head end and oesophagus in lateral view to show general shape.

Fig. 2.—Female tail, lateral view.

Fig. 3.—Anterior gonad showing cellular oviduct between uterus and ovary.

Figs. 4 and 5.—Male tail in lateral and ventral aspect respectively, showing shape of spicules and gubernaculum and distribution of bursal papillae.

In the light of this information Mr. Satchell made a closer examination of *Psychoda* flies and in due course reported that he had found the nematode larvae coiled round their bodies exactly as described by Bovien. It is clear from these observations that the larvae of *R. curvicaudata* are transported on the outside of the bodies of *Psychoda* flies which thus serve as agents for their dispersal.

Bovien found at least two species of *Rhabditis* associated in this manner with the flies he examined. One of these he described under the name of *R. dubia* n.sp. The other he did not name but recognised that it differed from *R. dubia* in having a remarkably long tail both in the larval and the adult stage. Both of these species are different from *R. curvicaudata* so that at the present time we know of at least 3 species of *Rhabditis* whose larvae are dispersed by being coiled round the bodies of flies belonging to the genus *Psychoda*. The association is a further instance of the now well established commensalism which has been proved to exist between certain nematodes of the genera *Rhabditis, Diplogaster* and *Cheilobus* and insects, including both beetles and flies as, for example, between *Aphodius fimetarius* (and various other dung beetles) and *R. coarctata* elucidated by Triffitt and Oldham (1927), and between *Drosophila confusa* and *R. pellio* described by Aubertot (1923). Bovien (l.c.) gives details of similar associations between various insects and certain species of *Diplogaster* and *Cheilobus quadrilabiatus*. In all cases the nematodes and the insects feed and breed in a common medium; the insects serving as the means of dispersal of the nematodes whose larvae become attached to them.

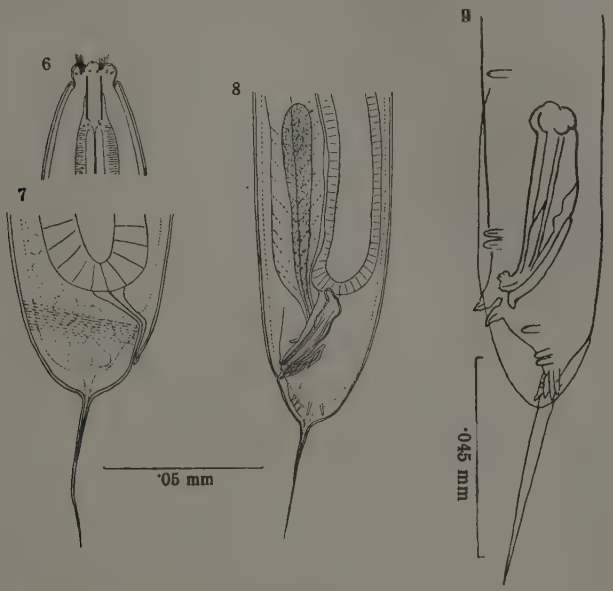
2. *Rhabditis paraciliata* n. sp.

Morphology.

Dimensions : *Female,* length, 0·76 mm. to 0·94 mm., $\alpha = 18$–19, $\beta = 4$–5, $\gamma = 15{\cdot}6$–19, V = 54·6%–56·7%, average of 8 specimens, length, 0·82 mm., $\alpha = 18{\cdot}6$, $\beta = 4{\cdot}5$, $\gamma = 17$, V = 55·3%. *Male,* length, 0·65 mm. to 0·86 mm., $\alpha = 15$–17, $\beta = 3{\cdot}6$–$5{\cdot}2$, $\gamma = 12$–14, average of 8 specimens, length, 0·75 mm., $\alpha = 16{\cdot}8$, $\beta = 4{\cdot}5$, $\gamma = 13$.

In general form and structure the adults of both sexes resemble those of *R. curvicaudata* and *R. ciliata* Fuchs in having a fairly stout build with a smooth cuticle and especially in having rounded, dome-shaped tail ends furnished with a fine terminal caudal process. The head (fig. 6) is offset by constriction and is capable of being withdrawn into the anterior end of the body. It is made up of 6 roundly conical lips each of which appears to carry 3 papillae, one forwardly and two rather laterally directed. Between the 6 lips there are tufts of fine hair-like bristles each group consisting of 5 or 6 forwardly directed bristles arising from an interlabial groove. In *R. ciliata* Fuchs shows bristles arising practically medially from the lips themselves and not restricted to the interlabial spaces. This is one point of difference

between the new species and *R. ciliata*. The stoma is typical consisting of a protostom about 15–16μ long by 5–6μ wide, followed by the telostom in which the telorhabdions appear as refractive dots. The oesophagus is well developed and has practically the same shape as



Rhabditis paraciliata n. sp.

Fig. 6.—Head end showing interlabial bristles.

Fig. 7.—Female tail in lateral aspect.

Fig. 8.—Male tail in lateral aspect, showing shape of spicules and gubernaculum, arrangements of bursal papillae and an ejaculatory gland lying over the terminal region of the vas deferens.

Fig. 9.—*Rhabditis ciliata* Fuchs, 1931. Lateral view of male tail showing spicules, etc., and distribution of bursal papillae, for comparison with fig. 8 (after Fuchs).

that of *R. curvicaudata*; the corpus leading to the slightly swollen median bulb which is followed by a rather long isthmus which ends in a large terminal bulb containing serrated valves. The nerve ring crosses the isthmus almost medially and the inconspicuous excretory pore lies just posterior to it on the ventral side.

Female. The tail end (fig. 7) is bluntly rounded and carries a fine tapering terminal caudal process, the core of which is formed by a prolongation of the body substance. The rectum opens very close to the rounded end of the body. The vulva is slightly post-equatorial in position. The gonads are paired, opposed and reflexed. Each uterus contains from 7–15 eggs at a time and sperms are located at the distal end of each uterus. There does not appear to be a distinct cellular oviduct between each uterus and its ovary such as is found in *R. curvicaudata.*

Male. The gonad is single and reaches well forward in the body with the anterior end reflexed for a short distance. The ejaculatory duct opens into the ventral floor of the rectum close to its commencement. On each side of the terminal region of the vas deferens there is a rather clavate, mononucleate ejaculatory gland which appears to empty into the cloaca close to the junction of the ejaculatory duct with the rectum. The paired spicules are about 29–30μ long. Each is cephalated anteriorly with a fairly prominent ventral knob. The shaft of each tapers a little to the tip which carries two rounded points when viewed laterally. The tip is not bent forward into two irregular hooks as in *R. ciliata.* The gubernaculum is about 20μ long and the inner end appears to be folded back on itself for a short distance. The rounded tail carries a fine terminal caudal process as in the female. The cuticle of the tail region is inflated to form a bursa which is not markedly winged but is rather similar to that of *R. curvicaudata.* There are 9 pairs of caudal papillae arranged as in fig. 8. 1 is a little preanal and is directed rather laterally, 2 and 3 form an adanal pair, 4 is ventro-laterally directed and is closely followed by 5, 6 and 7; 8 is posteriorly directed whilst 9 is postero-dorsally directed. This arrangement differs from that found in *R. ciliata* (fig. 9) chiefly in the position of No. 1 which in that species is situated far forward in advance of the heads of the spicules and also in the different relative distribution of Nos. 6, 7, 8 and 9. In *R. ciliata* there also appears to be a pair of very small papillae close to the base of the terminal caudal process. Whilst showing a general resemblance to *R. ciliata* the new species differs from it in its smaller size, in having the head bristles restricted to the interlabial furrows, in the shape of the spicules and in the distribution of the bursal papillae.

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A note on the feeding of the nematode, *Anguillulina macrura*.

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CERTAIN species of the nematode genus *Anguillulina* which commonly occur in close association with the roots of grasses are known to invade the root cortex and to reproduce therein. Thus *Anguillulina pratensis*, *A. obtusa* and *A. erythrinae* all occur in the roots of *Agrostis stolonifera* L. in pastures at this Institute. The writer has on a few occasions also found one or two specimens of the small, tapering-tailed species, *A. agricola* and *A. costata*, within the root cortex of the same kind of grass but further observations are needed before we can say definitely whether these two species actually parasitize roots and reproduce within the tissues. An occasional specimen of *A. intermedia* has also been found by the writer once or twice inside root tissues but never in what could be regarded as fresh, sound roots, and in view of Linford's (1937) observations on the ability of this species to feed upon fungal hyphae, it is probably safest to regard it as not a true parasite. The examples found may have entered partly decayed roots in search of fungal hyphae.

Concerning the feeding habits of certain other species which are sometimes obtained in Baermann extracts from turf such as *A. dubia*, *A. robusta*, *A. macrura* and *A. lamellifera* there is still much to be learnt, i.e. whether they are wholly parasitic or are merely intermittent feeders.

On one of these species, namely *A. macrura,* the writer can throw a little light as on two separate occasions it has been found attached to roots. The first time, an adult female worm was found with the anterior, oesophageal region of the body inserted into the cortex of the root of an oat seedling, in December, 1939. This worm was dissected out and finally mounted. In the same month, in a root of perennial rye-grass (*Lolium perenne* L.) which had been dug up from a garden plot and the roots stained with acid fuchsin, an adult male of *A. macrura,*

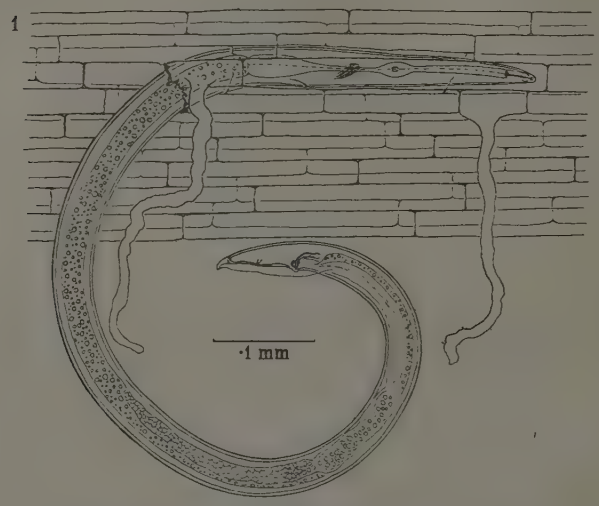

Fig. 1.—Small portion of root of perennial rye-grass (*Lolium perenne* L.) with oesophageal region of an adult male of *Anguillulina macrura* inserted into it.

1·32 mm. long, was found with the oesophageal region embedded in the cortex and the rest of the body lying outside amongst the root hairs. This portion of the root with the attached worm was cut out and, after suitably processing, was mounted in glycerine. Fig. 1 shows the whole worm and a small area of one side of the root but only two of the numerous root hairs have been drawn. It can be seen

that the worm entered the root by penetrating below an epidermal cell bearing a root hair. The wall here is broken and the oesophageal region of the worm lies outstretched about one cell deep beneath the epidermis. Two other transverse cell walls have been broken through.

Whether this species ever completely enters a root cannot be stated but, so far, the writer has never found this to be the case in numerous examinations of stained grass and seedling oat roots, and it seems quite possible that the two examples found with the oesophageal region inserted into roots represent its normal manner of feeding. If this is so then *A. macrura* may be regarded as a semi or intermittent parasite which partly penetrates a root for a time and then, withdrawing itself, lives freely in the soil until it feeds again in a similar fashion.

We find at least three degrees of parasitism represented by worms associated closely with grass roots. There are browsing organisms such as the small and very common *Paratylenchus macrophallus* (de Man) which live among the root hairs and feed by inserting only the mouth spear into root tissues. Then we have intermittent feeders such as *A. macrura* in which the oesophageal region of the body is forced into the cortex for a time with the rest of the body remaining outside the root. Finally there are the wholly parasitic species, such as *A. pratensis*, *A. obtusa* which feed and reproduce wholly within the root tissues whence they migrate to invade other roots.

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Journal of Helminthology, Vol. XXI, *No.* 1, 1943, *pp.* 20–21.

On the status of *Aphelenchus agricola* Maupas, 1900, with remarks on the genus *Paraphelenchus.*

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Recent re-examination of the paper by Maupas (1900) in which, on pp. 571-575, he describes *Aphelenchus agricola* de Man, and the figures 2-7 of plate 25 illustrating the same, have confirmed the writer in a view he expressed in an earlier paper (Goodey, 1928, p.135) that Maupas was correct in identifying the worms found by him in Algiers with *Aphelenchus agricola* de Man, 1881 = *Aphelenchus avenae* Bastian, 1865.

The reasons for this conclusion are the agreement in the size, shape and general build of the body, the bluntly rounded tail, the presence of 10 12 fine longitudinal striations on the lateral fields, the size and shape of the buccal spear without basal swellings and the possession of a post-bulbar region of oesophagus with the oesophageal glands lying dorsal to it and to the beginning of the intestine and not within its confines. The writer thus disagrees with Micoletzky's (1921) systematics where on p.603 he transferred *Aphelenchus agricola* of Maupas to a newly erected subgenus, *Paraphelenchus*, naming it *Aphelenchus* (*Paraphelenchus*) *maupasi* nom. nov.

Repeated examinations of fresh and mounted specimens of *Aphelenchus avenae* have satisfied the writer of the presence of a post-bulbar region of the oesophagus from one-half to two-thirds the length of the pre-corpus, much as depicted by Maupas (l. c.) and by the writer (1927) with the oesophageal glands lying outside it. There is no doubt, in the writer's opinion, that the worms drawn by Maupas were specimens of *Aphelenchus avenae.* Steiner (1942), however, appears to hold the view that the species is different from *A. avenae* and has named it *A. maupasi* (Micoletzky) n. comb.

Paraphelenchus, raised to generic rank by Micoletzky (1925), p. 248, differs from *Aphelenchus* in the following features :—In possessing a longer and more definite post-bulbar region of the oesophagus which is slightly swollen behind and is offset from the intestine but contains the oesophageal glands within its confines. In the shape of the female tail. In the male tail lacking a bursa and having its caudal papillae arranged differently from those of *Aphelenchus avenae* which has a well developed bursa.

de Man (1921) described and figured two male worms collected from sandy soil in the vicinity of Scheveningen, Holland, which he considered to be males of his *Aphelenchus agricola* de Man, 1881. There can be no doubt, however, that they are not males of this species but belong to the genus *Paraphelenchus* as revealed by the oesophageal characters and by the absence of a bursa. They differ from *Paraphelenchus pseudoparietinus* (Micol., 1921), Micol, 1925, in that the tip of the tail lacks a terminal process and the gubernaculum is larger than that of *P. pseudoparietinus* as figured by Micoletzky (1925). The latter considered them to be males of his so-called *Paraphelenchus maupasi*. This binomial, for reasons already adduced, is untenable and it therefore becomes necessary to give de Man's species a new designation. The writer, herewith, proposes the name *Paraphelenchus arenaceus* nom. nov. The specific name *arenaceus* is adopted because the worms occurred in sandy soil.

With the removal of *Paraphelenchus maupasi* (syn. *Aphelenchus agricola* of Maupas = *A. agricola* de Man, 1881 = *A. avenae* Bastian, 1865) from the genus *Paraphelenchus*, the latter is left with the three species, viz. :—

1. *Paraphelenchus pseudoparietinus* (Micol., 1921) Micol., 1925.
   syn. *Aphelenchus* (*Paraphelenchus*) *pseudoparietinus* Micol., 1921.
2. *Paraphelenchus amblyurus* Steiner, 1934.
3. *Paraphelenchus aranaceus* nom. nov.
   syn. *Aphelenchus agricola* de Man, 1921.

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Anguillulina dipsaci in the inflorescence of onions and in samples of onion seed.

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Disease in onions due to the stem eelworm, *Anguillulina dipsaci*, causing a soft rot of the bulb and a general stunting of the plant, commonly called "bloat," has been known for many years. Its symptoms and pathology were fairly fully described and figured by Bos (1888-92). More recently a considerable amount of investigation on the disease has been carried out in U.S.A., particularly by Newhall and Chitwood [see Newhall & Chitwood (1940), Chitwood, Newhall & Clement (1940), Newhall (1941) and (1943)]. It is not intended to discuss this work here but merely to indicate that our knowledge of the disease set up both in the seedling plant and in the grown bulb is now fairly full and detailed.

Certain gaps in our knowledge of the bionomics of the disease have, however, remained, particularly those relating to its sporadic incidence in areas where onions have not previously been grown or have not been grown for many years. One possibility which needed exploration was whether the parasite could be seed-borne. Bos (1889, p. 346) provided a note on this matter. He said that in seed harvested from a diseased crop of onions and sent to him in the spring of 1886, he was able on close examination to see that some of the seed contained eelworms but only in small quantity. When this seed was sown in clean soil 3% of the resulting seedlings were affected with typical symptoms. The infected seed could not be distinguished externally from clean seed. As far as the writer is aware, this is the only record extant of the association of the stem eelworm in a living condition with onion seed and Bos apparently did not publish anything further on the matter. It will be shown later that the parasite does not occur within the seed coat.

On the general subject of the dispersal of the stem eelworm by means of seed, it may be pointed out that in a number of different hosts there is well established evidence of the presence of the parasite in parts of the flower and in seed capsules. In the case of the common weed, cat's-ear (*Hypochoeris radicata*) Godfrey (1924) showed that the worms may actually occur within the seed coats. In other cases such as that described by Goodey (1937a) in affected plants of polyanthus (*Primula polyantha*) the worms were present in flower buds of the sepals, petals, receptacles and anthers. The writer has also found them

in quantity in infected inflorescences of *Primula japonica,* causing swelling of the seed capsules. In seed capsules of daffodils growing from eelworm infected bulbs, the writer found the parasites inside the seed capsules within the funicles or seed stalks but not within the seed coats. When such seed capsules were dried the parasite occurred in numbers in the débris accompanying the seed. Cobb (1929) showed that commercial samples of red clover and lucerne seed might, on soaking in water, yield living examples of the parasite. The purpose of the present paper is to put on record evidence showing that in a crop of onions planted for seed production the parasite occurred in large numbers in the tissues of many inflorescences and also in the tissues of the hollow flowering stems. In addition, evidence is presented showing that the parasite may occur in a viable condition in samples of onion seed drawn from commercial stocks.

PRESENCE OF PARASITE IN INFLORESCENCES AND FLOWER-STEMS.

On August 6th, 1943, the writer received from Mr. S. G. Jary, Advisory Entomologist of the South-Eastern Agricultural College, Wye, Kent, three complete flower stems of onion which had been grown in the vicinity of Chichester in an onion " seed " crop. Many of the flower stems in this crop, according to Jary's accompanying letter, had turned yellow prematurely and many flowers had failed to set seed. He reported that he had found numerous larvae of the small narcissus bulb-fly, *Eumerus sp.,* in some of the bulbs which were soft, and also an abundance of eelworms which he considered to be *Anguillulina dipsaci.* Though aware of numerous outbreaks of " bloat " in onions which had been reported up and down the country, this was the first occasion on which he had seen the disease in onion " seed " plants. The writer was asked for a determination of the eelworms and for any evidence of the stem eelworm being seed-borne.

Examination of the tissues from the base of one of the bulbs showed large numbers of *A. dipsaci* in the drying material. Portions of the three inflorescences were cut off and soaked in water in separate Petri dishes. The washings were examined two days later when two of them were entirely negative for eelworms, but in the third dish 6 or 7 specimens of *A. dipsaci* were found consisting of one or two adults and a few larvae of various sizes. As this material had been packed and sent through the post with the stems folded, there was a possibility that the eelworms found in the washings of the inflorescence might have been contaminants from the infected bulb bases. In reporting on

these findings the writer asked for a further series of samples of flower heads and stems; requesting, at the same time, that care should be taken in collecting and packing the material to ensure that no contact was made between such flower heads and bulbs as might be sent. On August 24th a further supply of material was received consisting of flower heads with attached portions of the flower stems and some pieces of the stem bases. In his covering letter, Jary explained that the flower heads had been gathered and packed separately from the basal portions and there was thus no possibility of the heads being contaminated by contact with the stem bases.

The inflorescences, many of which showed an abundance of aborted flowers, were labelled serially 1 to 17, and from each a portion, consisting of pedicels and seed capsules, was cut off and placed in a large Petri dish. The material was then covered with water and, after gently shaking, was placed in an incubator at 24°C. and left overnight. On the following day the water of each dish was examined for the presence of nematodes with the following results. In 10 out of the 17 preparations active specimens of *A. dipsaci* were found. In 6 of the 10 positive dishes they were present in large numbers; in the other 4 they were few in numbers, thus pointing to a lighter infestation. The majority of the worms were in the pre-adult, infective stage. One dish which contained no specimens of *A. dipsaci* had large numbers of the cephalob eelworm, *Panagrolaimus rigidus*.

It was clear from these results that the parasite was quite abundant in the inflorescence itself and further detailed examinations were then begun to determine its precise location in the various parts of individual flowers, pedicels, etc. Flowers were removed from an inflorescence which, on soaking, had shown an abundance of the parasite and were placed in glass capsules with a small volume of distilled water. After soaking for a short time they were carefully dissected with needles and the coiled nematodes were floated out from the seed capsules and receptacles. In the same way eelworms were easily teased out from the flower pedicels. In all cases the worms revived and became active in the course of an hour or so. This method of locating the worms was soon superseded, however, by another whereby the worms were stained *in situ*. Seed capsules and pedicels were removed and flooded with hot water so as to kill the contained worms. The material was then processed by one or other of the two following methods: (i) taking it through graded alcohols to 70% and then staining overnight in a

saturated solution of Scarlet R. in 70% alcohol plus acetone, by which the worms were stained bright red, see Goodey (1937), (ii) treating with strong Flemming's solution for about an hour, followed by washing in running water, whereby the nematodes were stained a blackish brown by the osmic acid of the fixative. Subsequent treatment in both cases consisted in passage through 70% alcohol followed by butyl alcohol and the final mounting of suitable portions in Euparal. As the material contained quantities of air bubbles it was found advantageous to carry out some of the processing under reduced pressure in a vacuum embedding bath. By this means most of the air was removed and the material, as finally mounted, was practically free from air bubbles.

Examination of the preparations revealed the coiled eelworms in considerable numbers in the soft tissues of the pedicels surrounding the central bundle, in the denser tissues of the receptacle, i.e. at the junction of the pedicel with the base of the flower, in the withered remains of sepals and petals and within and upon the walls of the three carpels forming the walls of the seed capsule.

The question then remained, how had the eelworms made their way into the tissues of the inflorescence? In order to settle this the tissues of the scape or flower stem were examined. The scape, like the onion leaf, is hollow throughout its length and in the almost dry condition in which they were received, it was found that the innermost layers of many stems had separated from the surrounding tissues as a thin papery layer a few cells in thickness and containing some of the parallel vascular bundles. Portions of this material were taken and, on placing in water, were found to yield large numbers of *A. dipsaci* after soaking for half an hour or so. Examination of fresh preparations under the microscope revealed the presence of large numbers of coiled eelworms which gradually uncoiled as they absorbed water, and afterwards swam about freely. Pieces of the thin papery material were removed, treated with hot water for a few minutes in order to kill the contained worms and then processed and stained either with Scarlet R. or with strong Flemming's solution as already described, followed by final mounting in Euparal. The resulting preparations show enormous numbers of pre-adult, infective larvae lodged amongst the cells. Such pieces taken from the upper, middle and lower parts of scapes showed the presence of the parasite throughout the entire length. Incidentally, it may be mentioned that there was no recognisable difference in the outward

appearance of those scapes which carried the parasite in such large numbers and those which were free from infection.

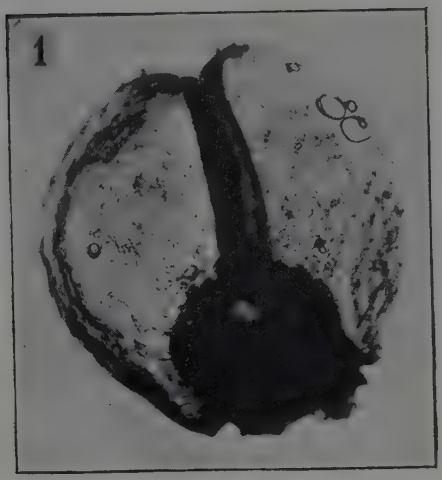
It will be realised that the infected material investigated does not provide data for establishing how the parasite becomes so extensively spread through the tissues of the scape and the inflorescence. Further investigations are being undertaken to determine the course of events in the infection of these structures and also to ascertain whether, in the case of onions grown for seed, the infestation of flower-stem and inflorescence arises from an already lightly infected bulb or from the surrounding soil.

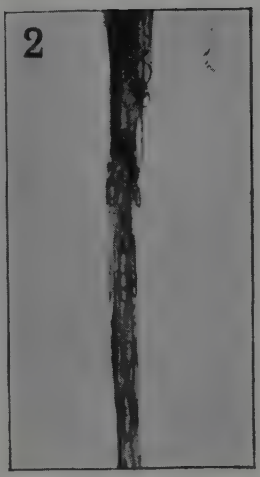
The following facts, however, seem to point to lightly infected bulbs serving as the source of infection. Enquiries made by Mr. Jary elicited the fact that bulbs from the same parent stock as were grown at the nursery near Chichester were also grown at a farm in Essex. In December, 1943, a ¼lb. sample of cleaned seed from this crop was received by the writer. It had every appearance of being a good clean sample and contained very little flower-part débris. When, however, it was soaked overnight in sterile distilled water, active infective larvae of the parasite were found in the washings on the following morning; thus proving that the parasite must have been present in the seed heads of this crop also. It further demonstrated that the ordinary processes of winnowing and cleaning had not removed the parasite from the seeds. Since the parent bulbs planted both in Sussex and in Essex gave rise to seed contaminated with the parasite, it may reasonably be concluded that, in all probability, the bulbs themselves were infected at the time of planting.

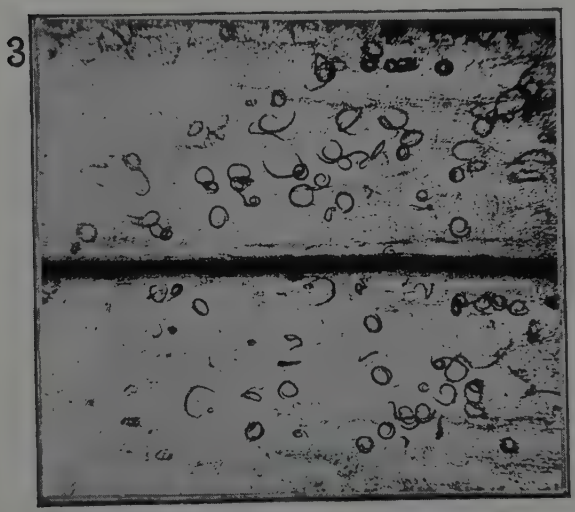
The foregoing observations establish the fact that at the time the seeds are setting the parasite may occur in large numbers in various parts of the flower and within the flower stalk. As these parts gradually dry out large numbers of the parasite in the infective stage coil up in and upon the drying tissues. Much of this forms small dry fragments which accompany the seed as it falls out of the seed capsules and the seed may thus carry the parasite on it or in the particles accompanying it.

Since the parasite may occur in enormous numbers in the dry flower stem and seed heads, a point of great practical importance arises in connection with the final disposal of such highly infective and dangerous material. It is obvious that it should all be very carefully collected and burned and should never be ploughed under or put into a compost heap.

So far as the writer has been able to ascertain, the parasite does not







Photomicrographs, under low magnification, of coiled, infective larvae of *Anguillulina dipsaci*, stained with Flemming's solution, in various parts of an onion flower tissue and stem.

1. On the wall of one half of a seed capsule. 2. In a portion of a flower pedicel. 3. In the tissues from the inside of a scape or flower stem.

To face p. 26.

become lodged within the seed coat. This point has been tested by the examination of seeds taken from within infected seed capsules and dissecting them under water with consistently negative results.

The fact that the parasite is a contaminant on the outside of the seed raises the question of whether it can be removed by the ordinary processes of winnowing and cleaning. The case has already been cited of it having been found in cleaned samples of the Essex-grown seed. Similarly it was found on the seeds of a sample of the Sussex-grown crop which had been very carefully hand cleaned for the writer by expert cleaners. It also occurred in considerable numbers in bulk samples of the same seed, ready for distribution to the trade.

Such contaminated seed is a source of great potential danger to the onion crop growing from it and as a measure of control some kind of treatment is necessary which will eliminate the parasite without injury to the seed. Experiments are already being undertaken by the writer to this end.

A. DIPSACI IN COMMERCIAL SAMPLES OF ONION SEED

The findings already dealt with naturally lead on to observations which the writer has made to determine whether living examples of the parasite may occur in commercial samples of onion seed. In order to test this matter arrangements were made whereby small samples of onion seed were received from the Official Seed Testing Station, National Institute of Agricultural Botany, Cambridge. These samples were portions of what remained over from batches of onion seed samples sent to the Station for seed-testing purposes by various seed merchants, and a series of 248 such samples were first of all examined. The seeds were from crops grown in 1941, and were sent to the writer under a serial identification number with no particulars as to variety or place of origin. Most of the samples weighed less than 5 grammes, but in a few cases there were up to 10 or 11 grammes of seed.

In order to examine for the presence of eelworms a quantity of 4 grammes was weighed out and placed in a clean Petri dish and then covered with sterile distilled water. Where a packet contained less than 4 grammes the whole of it was taken. In passing, it may be mentioned that there were roughly 1,000 seeds in 4 grammes. After gently shaking each dish so as to get the seeds wetted the dishes were left in the 24°C. incubator overnight and were examined on the following day The water of each dish was decanted into its respective lid which was then examined under a binocular microscope for the presence

of eelworms. Out of the 248 samples of seed examined in this way none was found to contain living or dead specimens of *A. dipsaci*. Twelve of the samples, however, were found which did contain a few specimens of such free-living eelworms as *Rhabditis lambdiensis, Rhabditis* spp. and *Panagrolaimus rigidus,* which in all cases, except one, were dead. The one exception was a single living example of *P. rigidus* which occurred in one dish along with a dead specimen of the same species.

At a later date a further series of 46 samples of British-grown onion seeds, also drawn from trade samples sent in for testing, were received from the Official Seed Testing Station. These were examined in exactly the same way as described above except that a 3 gramme sample was used instead of 4 grammes in each case. The results obtained differ from the previous ones in that 7 out of the 46 tested were positive for the presence of living specimens of *A. dipsaci*. In two of the samples motile infective larvae were present in fairly considerable numbers, i.e. 50 were picked up from the water of one dish and this was the highest number found. The next most numerous sample was 31 infective larvae. Of the 5 other positive samples one contained 7 infective larvae and the other 4 contained 1 or 2 each. The onion seed samples which proved to be positive for the presence of *A. dipsaci* were in no way dirtier or contained more obvious débris than those which turned out to be negative.

Further small quantities of seed were obtained of the three samples which had yielded respectively 50, 31 and 7 infective larvae on soaking in water. Three grammes of each were sown in separate seed boxes of partially sterilised soil on August 24th, 1943. Germination was even and the seedlings produced a good stand. Careful examination of the plants at various times up to 7 weeks after sowing failed to reveal a single affected seedling in any of the three boxes. It might reasonably be inferred from this result that, in the sample containing the highest number of infective larvae, i.e. 50 per 3 grammes of seed, the parasites were present in insufficient numbers to cause recognisable symptoms of disease in any of the seedlings. Unfortunately more seed of these particular samples was not available so the trials could not be repeated.

It would probably be unwise to attach too much importance to the results of a single experiment of this kind and further trials are obviously desirable to determine, as far as may be, the conditions under which small numbers of infective larvae can set up disease in onion

seedlings. Until the results of such trials are available it would be unsafe to conclude that the presence of even quite small numbers of infective larvae amongst onion seed is a matter of little practical importance since it is obviously desirable that so dangerous a parasite should be eliminated as completely as possible from any sample of onion seed offered for sale.

ADDITIONAL NOTE.

After the foregoing paper had been written, Mr. E. R. Wallace, of the Agricultural Institute, Kirton, Lincs., sent the writer particulars of an experiment carried out by him in which 4 different varieties of onions were sown in 3 replications in March, 1943. During the examination of the resulting crop, for the presence of a fungal disease, a number of eelworm infected bulbs were found in one of the varieties, one or two in another variety and none at all in the two remaining varieties.

A sample of the seed remaining over of the variety which had shown the greatest number of affected bulbs was sent to the writer and this was examined for the presence of the parasite. Three separate lots of seed, one of 4 grammes and two of 3 grammes each, were put up and soaked overnight in distilled water. The dishes were examined on the following day and good numbers of infective larvae of the parasite were found in each dish; 105 specimens in the 4 grammes sample and 40 in each of the 3 grammes samples. A sample of seed of the variety which had shown one or two affected bulbs was afterwards examined by the same methods but no specimens of the parasite were found. Seed of the other two varieties grown was not available so these, unfortunately, could not be tested.

Had the parasite occurred in the soil of the plot to begin with it is reasonable to suggest that all four varieties of onions grown would have shown infected bulbs; since most varieties of onion are susceptible to attack. Such bulbs, however, were most numerous in that variety amongst whose seed later examination revealed the presence of the parasite. It seems reasonable to infer, therefore, that, in all probability, the parasite was introduced along with the seed and in due course gave rise to disease in the bulbs.

ACKNOWLEDGMENTS.

The writer desires to thank Mr. S. G. Jary, Advisory Entomologist of Wye, for his great help in securing and sending the affected material from the onion " seed " crop which proved so interesting. Thanks are also due to Mr. C. C. Brett and Mr. E. G. Thompson, of the Official

Seed Testing Station, National Institute of Agricultural Botany, Cambridge, for their kindness in providing samples of onion seed, and to Mr. E. R. Wallace, of Kirton, for the seed and information which he provided.

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Tulip bulbs attacked by *Anguillulina dipsaci*.

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INTRODUCTION.

EELWORM disease of tulips was first reported by Bos (1906) in bulbs of the varieties " La Reine " and " Yellow Prince," grown at Sassenheim in Holland. Westerdijk (1906) dealt a little more fully with the same outbreak, described the typical brown ring symptoms shown by the bulbs and reported on a few preliminary trials designed to determine whether the parasite belonged to the same biological race as that attacking hyacinths.

Theobald (1912) reported the presence of *Tylenchus devastatrix* in a number of tulip bulbs (variety unnamed) which had been sent to him from Dublin, Ireland. In a later note Theobald (1924-25) speaks of a tulip grower in Thanet, Kent, having such bad attacks of eelworm in his bulbs that he decided to remove his nursery to clean ground. Whether the stem eelworm was actually found in affected tulip bulbs was, however, not stated.

The next record of the occurrence of the parasite in tulip material is that of Grace Sherman Cobb (1937) who reported the presence of the parasite in leaves of the variety " Le Notre " at Babylon, New York, U.S.A. The plants were volunteer bulbs growing in a plot in which eelworm infested narcissi had been growing. No special symptoms of attack were noted and only three leaves out of 16 examined contained the parasites.

More recently there has appeared a short paper by Chitwood & Machmer (1942) in which an outbreak of disease caused by the stem eelworm has been established in two varieties of tulip, viz., " Pride of Haarlem " and " Telescopium." In March, 1941, about 4,000 bulbs of " Pride of Haarlem," which were being forced in a greenhouse at Babylon, New York, were found showing split leaves and corollas as well as stunted and twisted flower stems. Enquiry showed that the bulbs had been acquired from a grower in North Carolina in the autumn of 1940. Stocks of bulbs of the same variety were later examined growing in the open at the establishment of the grower in N. Carolina and these too were found to be heavily infested with the eelworm. The grower considered that the bulbs were already infested on importation from Holland in the autumn of 1939. Part of the same stock of bulbs had been sold to a grower in Maryland and on being inspected, these also were found to be infested with the parasite.

In the case of the other variety " Telescopium," numbers of bulbs which were being forced in a greenhouse at Babylon, N.Y., were found to be attacked and the grower was of the opinion that the stock was infected at the time of importation from England in the autumn of 1941.

THE DISEASE IN ENGLAND.

Late in November, 1943, the writer received from Mr. J. C. F. Fryer, Director of the Ministry of Agriculture Plant Pathology Laboratory, Harpenden, a small parcel of diseased tulip bulbs which had been sent in for examination by Mr. J. Oldnall Page, one of the Ministry of Agriculture Inspectors stationed at Spalding, Lincs. Mr. Page reported that a bulb grower in his district had been compelled to destroy some thousands of tulip bulbs of the two varieties " Telescopium " and " Rynland " because they were so badly attacked by eelworm.

Examination of these bulbs showed that many of them were in an advanced stage of disease. When cut transversely they manifested typical brown-ring symptoms whilst others, not so severely affected,

showed a rather yellowish discoloration of the bulb scales. In many of them the basal plate was quite rotten and shrivelled and in one or two such cases there occurred small masses of eelworm " wool " on the underside of the basal plate. One such mass, on placing in a drop of water, was found to consist of some hundreds of the quiescent infective larvae of the parasite. On removing the brown tunic from the outside of the bulbs several of them were found to show a dirty yellowish discoloration with the surface of the outermost scale raised into small, flattish pustules many of which were more or less confluent. The parasite was found on teasing portions of this affected tissue in water. There can be no doubt that, although many of the bulbs were in an advanced state of disease with the tissues swarming with bulb mites, the primary cause of the disease was the nematode *Anguillulina dipsaci.*

So far, of course, the writer has not had an opportunity of seeing the symptoms of disease produced in the foliage and the flowers but there is every possibility that, in the spring, specimens of affected bulbs taken from the field will become available when it is hoped further observations on the symptoms manifested by the growing plant may be made.

It is of interest to note that the records of *Anguillulina dipsaci* on tulips are not limited to one or two varieties only but are found in varieties belonging to different groups. According to the Royal Horticultural Society's publication " *A classified list of Tulip names* " issued in 1939, the varieties " La Reine " and " Yellow Prince " belong to the Single, Early Flowering group. " Le Notre " and " Pride of Haarlem " are both Darwin tulips whilst " Telescopium " and " Rynland " belong to the group known as Triumph tulips. These are hybrid tulips of Dutch origin probably arising from a cross between an Early Flowering variety and either a Dutch Breeder tulip or a Cottage variety or a Darwin variety.

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Stem eelworm in onion bulbs, probably seed-borne in origin.

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On 23/3/43 a small experiment was sown with commercial onion seed at Kirton, being one of a series laid down at county centres in order to study the effect of different times of lifting on the development of wastage in store. Four varieties, which may be designated A, B, R, U, were sown in strips of three rows 10in. apart, in triplicate, the rows being 41 yards long and forming three randomised blocks. The liftings were also in randomised block pattern, 3 x 3, across the length of the rows. Two sub-plots of each variety were taken for convenience, consisting of adjacent six-foot lengths across the three rows, the produce of each sub-plot being put into a separate box immediately on lifting.

The dates of lifting and of transfer to indoor storage were as follows: —(1) Lifted, 26-27/7/43, transferred, 17/8/43; (2) Lifted, 16-17/8/43, transferred, 7/9/43; (3) Lifted, 6/9/43, transferred, 27/9/43.

Three distorted plants were found in the field at the first lifting, and one at the second, all being in the variety U. The boxes were left on the plots from which the onions had been lifted until the transfer dated given above. They were then taken to an unheated tulip store where the boxes were stacked in tiers of twelve, corresponding to the sub-plot strips across the rows. Apart from being in separate tiers, the members of each pair of sub-plot boxes were treated alike.

The first examinations and weighings were carried out at various times between 1/10/43 and 19/10/43, the aim in view being to record the incidence of *Botrytis* spp, of " other fungi " as a single group and of mechanical damage, with any combinations of these. The first box of variety U examined contained some soft bulbs with a different " feel " from that of onions softened only by neck rot. On the second day of weighing, a single bulb with eelworm " wool " was found. The incidence of softening was then recorded as a separate classification, sometimes in combination with fungus attack, and completely wet bulbs were also encountered. Representative material from affected boxes was kept and taken to the laboratory, and specimens were found to contain living eelworms as well as numerous eggs. Suspected material, afterwards confirmed as having been attacked, was not

uniform to the naked eye, and a number of different external appearances could be distinguished, excluding those bulbs which had become partly liquefied or attacked by a fungus.

All these bulbs were well finished off at the neck but presented the following different pictures :—

(*a*) Bulb of normal shape and hard. Arising from the base a dead-white area, not coinciding in outline with the previously overlying scale, and separated from the straw coloured or pale brown area by a more deeply pigmented border from 1–4mm. wide.

(*b*) Bulb of normal shape. Outermost fleshy scale softened and somewhat pliable, no bordered lesion.

(*c*) Bulb puffy. Affected part of outermost fleshy scale dead-white and projecting slightly as a longitudinal segment, with obvious thickening and change of texture, occasionally creased or puckered away from the next underlying scale.

(*d*) Bulb as in (*c*), but cracked longitudinally. Various stages from cracks 2–3mm. to full length of bulb have been noted.

(*e*) Bulb as in (*c*), but bearing also short raised ridges 1–5mm. × 1mm. projecting from the general surface of the scale, elongated in the direction of the vascular strands and lying between them.

(*f*) Bulbs as in (*a*), (*b*) or (*c*) but with eelworm " wool " either (i) on the surface of an equatorial lesion, or (ii) extruded from the base, often in the form of snake-like threads at the edge of the basal plate where fresh root initials were growing through, or (iii) in larger masses at the base, the plate having been forced away to one side, like a lid, owing to the hypertrophy of the innermost tissues. (This is comparable to the state of affairs seen in narcissus, where the larger basal plate often becomes completely detached like a cork from a bottle.)

It may be emphasised here that many of these bulbs were undistinguishable by eye from healthy ones except under very close scrutiny, and attention was first drawn to them only because the bulbs were being " handled " as an aid to the discovery of hidden neck-rot.

When all the boxes had been examined once, it was found that the softened bulbs attacked by fungi and the wet bulbs were almost entirely confined to boxes containing also a number of bulbs in classes (*a*)—(*f*), and a preponderance of specimens in these latter classes was found to be associated with variety U.

The totals of " soft " bulbs per cross strip at the first sorting, arranged according to variety, were as follows :—Var. A, 0, 3, 2;

Var. B, 0, 0, 0; Var. R, 3, 12, 0; Var. U, 64, 33, 50. The number of bulbs on which eelworm was recognised was as follows :—Var. A, 0, 0, 0; Var. B, 0, 0, 0; Var. R, 1, 1, 0; Var. U, 7, 6, 4.

No seed of varieties A or B was available, but a sample of the seed of variety U was sent to Dr. T. Goodey, and later at his request a sample of variety R.

We are indebted to him for permission to quote his findings :—

Variety U. " An examination of 3 weighed samples of the seed was made : two of 3gm. each and one of 4gm. each being soaked overnight in a separate dish of sterile distilled water. From the 4gm. sample, 105 specimens of *Anguillulina dipsaci* were obtained and from the other two about 40 specimens each. Most of the worms collected were living and active in water and in the pre-adult infective stage."

Variety R. " Four dishes each of 4gm. of the seed were soaked in distilled water. Not a single example of the worm was found in any of them."

These, in conjunction with the other figures, seem to offer reasonable circumstantial evidence for the facility of distribution of this pest through the medium of a contaminated sample of commercial seed. An alternative would be to suppose that there was no connection between the seed contamination and subsequent findings, but that variety U was very much more susceptible to eelworm attack from some general source to which the other varieties were also exposed. Any hypothesis of susceptibility of U at a special stage in relation to its maturity, by contrast with the stages reached by the three different varieties at some favourable time, would seem to be ruled out in advance by the three dates of lifting, since the soft bulbs were well distributed among the produce of the three different liftings. At present there is no evidence available to support the proposition of any marked inherent differences in susceptibility as between these varieties.

If the distribution of this eelworm by contaminated seed is accepted, we have here an example of the first season's outcome of this in a widely distributed attack of low intensity and late development. On such a basis it is possible to formulate an interpretation of certain cases encountered in the field, that are otherwise difficult to understand.

In 1942 more than one instance was met with in which the infestation was bad, leading to " wiping-out " of the plants over extended areas, on plots of land that had also carried onion in 1941 but not previously. The growers had noted few or none of the typical distorted plants in

1941, the plots having been satisfactory by commercial standards in the first year.

Such infestations might be supposed to derive from previous cultivated hosts, from tolerant weed hosts, or from contaminated seed. Accounts of the previous cropping were not illuminating in any of these cases and it was not possible to associate them with any special culture of hosts associated elsewhere with eelworm strains attacking onion. Contrary to expectation the outbreaks seemed to be practically confined to areas outside those devoted to narcissus culture. In addition, the oat crop had been a very minor feature of the agriculture of this district during 1930-1939, being grown on farms solely for horse feed. As may be imagined, oats was not a smallholder's crop, yet by far the majority of the eelworm cases encountered were on small plots of $\frac{1}{4}$-1 acre.

It may now be suggested that the use of contaminated seed could have been followed by the appearance of only a small number of distorted bulbs in the growing season, together with a much larger number of infested mature bulbs, not recognisable as such at normal lifting time. Some of these would be discarded from the windrows or the boxes (outdoors) when the onions were being bagged for sale in October. Such bulbs would not be liable to be recognised as victims of eelworm by the sorters, and cracked ones that had remained outdoors would also tend to be attacked by fungi, including *Botrytis allii*.

Such simple measures of plant hygiene as removing the diseased material from the field are not yet accepted as necessary by the growers and tend especially to be neglected in war-time. Thus one of us (ERW) has information of diseased onion bulbs being " ploughed in " to the land on which the crop was raised in 1943. Under such conditions, which are widespread, it is possible to understand the reappearance of the infestation in devastating form in a second season, but deriving from seed-borne infestation of the previous year. In advisory work the writers have stressed the danger involved in growing onions in succession, deriving their justification from the very severe cases of disease actually encountered.

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A Refinement of Gemmell's single cyst technique.

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Whilst studying the hatching of larvae from cysts of *Heterodera rostochiensis* in different samples of potato and tomato root excretions, in the hope of ultimately developing a technique for the standardisation of root excretion, the author felt that it might be worth while to publish details of a refinement of Gemmell's (1940) single cyst technique.

The Modified Single Cyst Technique.

A difficulty confronting workers who are seeking to investigate hatching phenomena in a quantitative manner has always been the very high inherent variability exhibited by different cysts, even when all are taken from the same sample. A very few experiments convinced the author that the only practical way of overcoming this difficulty and obtaining results amenable to statistical analysis, was by relating all hatches to separate individual cysts. While at first sight, single cyst hatching appears to involve a great deal of unnecessary work and labour, it was in fact found to be more precise and more economical in cysts than the use of any samples containing more than one cyst. Moreover the routine work involved using the technique to be described was not in the least arduous or laborious. In the author's opinion less work was involved than by the use of multi-cyst samples. From a statistical point of view the advantages of a single cyst technique are obvious, the increased degree of precision of all parametric estimates follows naturally on the increase in the number of degrees of freedom on which each estimated parameter is based.

Gemmell's original single cyst technique (Gemmell 1940) consisted of placing individual single cysts in drops of root excretion on coverslips which were supported on glass rings in 12cm. petri dishes. The surrounding atmosphere was kept moist by means of a damp filter paper placed on the floor of the dish and punched with half-inch holes in which the glass rings rested. Gemmell recorded that little or no evaporation of the root excretion occurred.

In the hands of the author certain disadvantages of this technique became apparent. Each coverslip had to be removed prior to counting of the escaped larvae, in order that condensation moisture could be

wiped from the under surface; while it was frequently found that condensation of water vapour on the upper surface caused very serious dilution of the root excretion, thus introducing a serious but unpredictable error. Moreover, the number of single cysts which could be put up in any one Petri-dish was very small (Gemmell suggests five per dish), while the number of cysts which had to be put up to form a single series was at least fifty; consequently, in order to obtain a result that was at all precise a very large number of Petri dishes had to be used—a very undesirable state of affairs where incubator space is limited.

An improvement on the Gemmell technique was suggested by Ellenby (Ellenby 1943). He suggested stamping vaseline rings on the floor of the Petri dish and placing in each a single cyst contained in a drop of root excretion. The chamber is maintained in a moist condition by means of filter paper which adheres to the lid of the dish.

Ellenby claims that using this modification 25 cysts could be accommodated per Petri dish and that condensation difficulties were overcome. This technique was found to have certain advantages over Gemmell's but was still unnecessarily troublesome, the use of vaseline was messy, quite apart from the fact that one is not able to rule out the possibility that it might contain some water-soluble impurity which could affect the root excretion. It was also essential to examine each dish daily since if left for a longer time, the filter paper frequently dried sufficiently for the humidity to fall within the chamber with consequent evaporation and concentration of the root excretion within the rings. The use of paraffin and other waxes in place of vaseline was tried and proved equally unsatisfactory.

In considering the problem in so far as it has been presented one may recognise the following aspects :—

(*a*) The maintenance of a uniformly humid atmosphere over a reasonable period of time.

(*b*) Securing a reasonable volume of root excretion per cyst in order that concentration changes resulting from evaporation or condensation shall be as small as possible.

(*c*) The reduction of manipulation and handling to a minimum.

The first difficulty may be overcome quite simply by using a rectangular sheet of glass cut to such a size as to fit within a Petri dish. Any form of rings or cells whether they be of glass, paraffin wax, vaseline or other substance may then be fixed on this sheet. The sheet of glass is then supported in the Petri dish on strips of glass tubing

leaving a space below the plate for a reservoir of distilled water. At the side of the dish is placed a strip of filter paper, one end dipping into the water, the other end overhanging the edge of the dish. Moistened filter paper is stuck to the roof of the dish so it will be seen that when the lid is placed in position, the strip of filter paper having its upper end in contact with the upper sheet, will act as a wick and keep it moist without the necessity for constant examination. As a test such set-ups have been left in the incubator at 24°C. with no attention for over three weeks, at the end of which time the upper filter paper was still quite moist.

In order to increase the quantity of root excretion used per cyst and to overcome the various difficulties introduced by the use of vaseline and wax, the author devised a method of making small glass dishes or cells which could be cemented onto the glass plate with Canada Balsam. These dishes may be made from glass coverslips $\frac{5}{8}$ in. diameter, No. 1 gauge. The only apparatus needed is a carbon rod taken from a unit dry cell battery, Drydex T20 or Eveready U2 being suitable. The centre carbons from such batteries measure approximately $2\frac{1}{4}$ in. long by $\frac{5}{16}$ in. diameter. After removal from the cell the end of each carbon is rubbed down to a circular point on coarse emery paper, the angle of the point being approximately 45°. The pointed end is then rubbed down leaving a circular area $\frac{3}{16}$ in. diameter. (Fig. 1.). The carbon is clamped vertically, tapered end upwards in a retort stand, and heated in the blue flame of a bunsen until all the moisture and wax in it is removed or burnt away. It is then ready for use.

A glass cell is made by placing a coverslip centrally on the top of the rod and heating it from above in a blue bunsen flame. In the heat of the flame, the edges of the coverslip will melt and collapse over the carbon to form a small flat-bottomed dish resembling in shape a miniature basin, which can be removed from the carbon with forceps. Provided reasonable care is taken such dishes are quite even in shape and hold approximately 0·2ccs. of fluid. Very little practice is necessary in order to make these dishes at the rate of one hundred per hour, while the number of failures due to breakages need only be very small. Since there are approximately 180 coverslips to the half ounce it is reasonable to expect at least 160 dishes per half ounce of coverslips.

In use the dishes are mounted on a glass sheet measuring four inches square. Two adjacent corners of this sheet are cut away in order that the glass sheet shall fit into the base of a 15 cm. Petri dish. Plates of these dimensions can accommodate 50 cells in six rows of eight each, the rows running parallel to the side without cut-away corners. There will then be sufficient space left for the remaining two dishes to be placed in the seventh row. Orientation of the large slide thus formed and identification of individual cells then becomes possible without the necessity of actually numbering individual cells. The balsam used for cementing the dishes to the glass is dried in an oven in the usual way and a permanent piece of apparatus is thus secured.

Cleaning of the slide is accomplished by washing under the tap, using a soft brush and " Vim " for removing any obstinate stains or deposits, rinsing in distilled water and drying in a cool oven. If necessary a bichromate-sulphuric acid mixture may be used, each cell being filled with this mixture and the slide allowed to stand overnight with subsequent thorough rinsing and drying. Should any one of the cells become broken or damaged it is a simple matter to remove it using the point of a hot scalpel and replacing a new cell, again cementing with balsam.

For examination all that is necessary is to remove the slide from its dish and wipe the underside with a cloth. With some binocular microscopes difficulty may be experienced when examining, due to the stage of the microscope being too small to accommodate the slide without fear of its falling off when cells near the ends are being examined. This may be obviated if a small drop of water or glycerine be placed on the centre of the stage and a large sheet of glass be pressed on it.

The glass will then be found to adhere firmly to the stage and form a new and larger stage sufficient in extent to take the slide.

The advantages of this slide are many and need barely be enumerated. The comparatively large volume of fluid in each cell minimises errors due to changes in concentration consequent on evaporation or condensation; the large reservoir of fluid in the base ensures the maintainance of a humid atmosphere over long periods of time—a distinct advantage if only weekly or fortnightly examinations are necessary; the set-up is highly stable, for test purposes slides have been inclined to angles up to and including 30° from the horizontal without loss of fluid from the cells; the cells themselves are very strong since the rim of each thickens when being made; examination is easy and change of fluid can be expeditiously accomplished by means of a fine pipette.

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Note on the Use of Picric Acid as a Hatching Agent.

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When carrying out hatching experiments it would frequently be an advantage to have a quantitative measure of the activity of the root excretion used and search was accordingly made for a stable substance which could be made to a solution of constant known strength and which could be used as the basis for the comparison of the activity of different samples of root excretion. Hurst (Hurst 1937) records that tyrosine in dilute solution, lysine in the form of its picrate as well as picric acid at ·01% concentration caused active hatching to occur and experiments using the above single cyst technique were conducted to investigate these claims. The results with the former two substances were disappointing but dilute picric acid solutions were found to be very active. Since it is hoped to publish a further paper in which the effects of picric acid will be considered quantitatively and in detail it is not proposed to publish data in this article but concentration experiments showed that picric acid is most active at ·02—·01% con-

centrations, the solutions being made up in distilled water. Several experiments were conducted in which picric acid in these concentrations was compared with water and with root excretion; in all cases picric acid hatches were significantly greater than water hatches although picric acid was not generally as active as root excretion taken from vigorously growing plants. On the other hand at the time of writing (late December) when plant growth is slow, picric acid is usually superior to root excretion. As far as can be made out at present the effect of picric acid is similar to that of root excretion in that the periods of maximum hatching appear to coincide more or less with those in root excretion. Moreover when different samples of cysts were subjected to the same root excretion and to picric acid a positive correlation was apparent between hatches in picric acid and in root excretion.

Experiments are in progress on the use of picric acid as an agent for use in the standardisation of root excretion and it is hoped to publish a further paper on this subject. The value of picric acid as an agent capable of inducing hatching has already been proved at this Institute, when during an experiment which was being conducted for Professor Leiper, tests on the hatchability of cysts using root excretion met with negative results due to the poor quality of the root excretion produced by plants in mid-winter; cysts, which failed to hatch in the root excretion provided, hatched on transfer to picric acid. In fact, it would be safe to say that in winter and late autumn, when root action is slight, picric acid is a more reliable hatching agent than is root excretion, unless very stringent precautions are taken to ensure that the plants from which the excretion is taken are in a state of active vigorous growth.

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Journal of Helminthology, Vol. XXI, *Nos.* 2 *and* 3, 1945, *pp.* 43–44.

Symptoms of disease in tulips caused by *Anguillulina dipsaci.*

By T. Goodey, D.Sc.

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A brief description has been given in an earlier paper (Goodey, 1943) of damage to tulip bulbs caused by the stem and bulb eelworm, *Anguillulina dipsaci.* In April, 1944, an opportunity arose, through the good offices of Mr. J. Oldnall Page, of Spalding, Lincs., of visiting a field in his district, in which eelworm-affected tulips were growing. Two of the varieties inspected were in flower and the other two were in an advanced bud condition. All four varieties exhibited marked symptoms of attack, and in the case of two of them in which there were a considerable number of plants, the damage caused to the blooms would entail a distinct financial loss to the grower. The largest planting consisted of the variety " Rijnland," the next largest was the variety " Wonderful." In addition to these there were smaller plantings of " William Copland " and " Mrs. Farncombe Sanders." In all four varieties evidences of attack were apparent on leaves, stems and flowers and, in general, the writer can confirm the findings of Chitwood and Machmer (1942) on the symptoms manifested by the growing plant. They mention longitudinal splitting of leaf, flower-stem and corolla. It may be well, however, to attempt a more detailed description of the chief symptoms and types of lesion shown by affected plants: see also the two photographs accompanying this paper.

Leaves.—Affected leaves are often irregular in shape with unequal areas of the leaf-blade on either side of the midrib which may be curved instead of straight. The blade itself often shows cream or yellowish stripes from a few millimetres in length to much longer, giving the leaf a rather blotched, unhealthy appearance. The striped areas cause a weakening of the leaf tissues with the result that slits and rents occur which often give the leaf the appearance of having been irregularly torn. The edges of the rents become withered and discoloured. Spickles with a distinctly swollen local thickening, such as are found on affected narcissus leaves, do not occur, but an affected leaf when passed between finger and thumb, may feel rather irregularly thickened.

Flower Stem.—The flower stem may exhibit a wide range of symptoms from a few local lesions without much thickening to considerable stunting and thickening with extensive lesions. In badly affected plants the stunting and thickening is most noticeable from the area of the insertion of the uppermost leaves to the flower itself. The region of the attachment of these leaves to the stem is often badly torn as a result of the lesions produced here (see fig. 1). The stem itself may be so thickened and stunted that there is failure to carry the flower bud upwards. Many flower stems show lesions in the form of local infections of the cortical tissues causing the epidermis over the affected regions to have a rugose or blistered appearance. Here and there such lesions become confluent and the epidermis splits or becomes cracked. Such lesions are often pale green or yellowish in colour and where they occur on one side of a stem only they give rise to marked irregularity in growth. This is particularly noticeable where such lesions occur close to the base of a flower as by the unequal elongation of the tissues on either side of the stem, the flower itself is forced out of the upright position and may appear with its tip twisted to one side or even inverted (figs. 1 and 2).

Flowers.—Lesions in the bases of flowers are commonly found with the result that the elements of the perianth are deformed; sepals and petals remaining deformed and discoloured. In some cases a flower may show a single discoloured spot or scar at the base of a petal or sepal giving it a blemished appearance without, however, causing any splitting of organ, but spoiling its symmetry and beauty.

When small portions of affected tissues from lesions on leaves and stems or from flowers are teased up in the water and examined under the microscope specimens of *A. dipsaci* can readily be found together with eggs and larvae, and there can be no doubt that the parasite is responsible for the pathological conditions manifested by the tissues.

Of the four varieties of tulips found to be infected, three, viz. :— " Wonderful," " William Copland " and " Mrs. Farncombe Sanders " are new varietal host records for the disease. " Wonderful " is a Mendel tulip, whilst " William Copland " and " Mrs. Farncombe Sanders " are both Darwin tulips.

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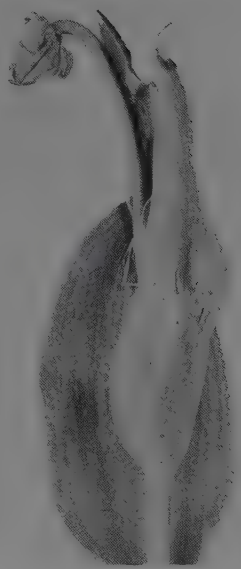

Photographs of two affected tulip plants showing the chief symptoms of disease due to *Anguillulina dipsaci* in leaf, stem and flower.

Fig. 1.—A plant of "Wonderful." Fig. 2.—A plant of "William Copland."

To face page 44.

Journal of Helminthology, Vol. XXI, *Nos.* 2 *and* 3, 1945, *pp.* 45–59.

Anguillulina dipsaci on onion seed and its control by fumigation with methyl bromide.

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In a recent paper (Goodey, 1943) it has been shown that the stem eelworm, *Anguillulina dipsaci*, may parasitize the inflorescence and flower stem of the onion and that as the seed ripens in the drying flowers, the parasite occurs on the seed itself, on the walls of the seed capsules and in various dry fragments of the flower parts which often accompany the seed in the roughly thrashed condition. In the same paper it is also shown that winnowing and cleaning such seed, even when very carefully done so as to eliminate all dry flower-part fragments, does not get rid of the parasite which remains attached to the seed.

Observations are now presented on the location of the parasite on the seed and a method of fumigation is described whereby seed can be successfully disinfected with complete destruction of the parasite but without injury to the seed.

Location on Onion Seed.

The presence of *A. dipsaci* on cleaned onion seed can be readily established by the examination of the water in which a given sample of seed has been soaked for several hours. The method usually adopted by the writer is to soak a few grams of onion seed (4 to 10 gms.) in water overnight in an incubator at 24°C. Any living worms attached to the seeds absorb water and revive, and can be found by examining the water under the microscope. With a lightly infected sample it is advantageous to spin the water in a centrifuge and examine the deposit from the bottom of the centrifuge tubes. In this way the writer has collected, in one case 45 worms from 11·1 gms. of seed, in a second case, 11 worms from 8 gms. of seed, and in a third case, 12 worms from 15·65 gms. of seed; all very light infections.

Such gross methods, however, tell us nothing about the actual distribution of the parasite among the seeds comprising a sample, nor do they yield any information about the location of the parasite on the seed. In order, therefore, to obtain more precise knowledge on these points the following procedure was adopted whereby separate lots of 1,000 onion seeds from two infected samples were examined individually by means of Fenwick's small culture cells (Fenwick, 1943). These

cells consist of small circular glass cups about 1 cm. in diameter by about 0·4 cm. deep. A battery of 50 of them, arranged in rows, is cemented to a sheet of glass which is suitably cut so as to fit into a Petri dish 15 cm. in diameter. For each sample of seed tested, 20 such dishes of 50 cells were eventually set up with a single onion seed in each cup containing a small quantity of sterile distilled water. The dishes were left in the incubator overnight and examined cell by cell on the following morning. Any cell in which nematodes were present was easily recognised and the number of living worms associated with a given seed could be readily counted.

1,000 onion seeds from a crop grown in Essex in 1943, and known to be carrying the parasite, were first tested in the above manner with the result that 17 only out of them were found to be carriers. The number of living nematodes associated with each of the 17 carriers was as follows :—47, 19, 10, 9, 8, 6, 6, 5, 4, 3, 3, 1, 1, 1, 1, 1, 1. This gives a total of 126 worms borne by only 17 seeds out of the 1,000. A similar test was made on 1,000 seeds from an onion crop grown in Sussex, from the same stock of parent bulbs, and also known to be carrying the parasite. In this case 12 seeds only were found to carry the parasite, the actual numbers of living worms per seed being as follows :—28, 19, 17, 6, 6, 4, 3, 1, 1, 1, 1, 1. In this case the total of 88 living worms was carried by 12 seeds.

Occasionally a worm or two can be seen still attached to a seed when examined by the above technique and it always appears to occur in the vicinity of the hilum, i.e. at the scar formed where the short funicle of the seed separates from its point of attachment to the placenta of the flower. In many infected onion flowers dissected by the writer the tissues of the placenta have been densely crowded with worms, and it is thus easy to understand their presence in the minute fragments of such tissue as remain attached to the seed at the hilum.

If we picture the random scattering of the 17 carrier seeds among 1,000 seeds sown, we can form some idea of the probable incidence of disease among the resulting seedlings. Lacking, for the time being, precise information as to the numbers of the parasite necessary to set up manifest symptoms of disease in an onion seedling, we may suggest, nevertheless, that the seedling arising from the seed carrying 47 infective larvae would probably become quite heavily parasitized. Similarly those seedings arising from seeds carrying 28 and 19 infective larvae might be more or less seriously affected, and thus as we descend

to those seeds bearing fewer numbers of the parasite we can assume that the resulting seedlings will be less heavily parasitized. The final 6 seeds, each with only one attached larva, might remain free from infection since all six of the worms might turn out to be males, and even if they happened to be females it would be essential for them first to be spermatized by a male before they could set up an infection and multiply in an onion seedling. It would seem, therefore, that it is probably from seeds carrying small numbers of infective larvae that infestations of light intensity arise which may manifest themselves quite late in the growing season or only during storage of the ripened bulbs, as found by Wallace and Wood (1943).

Destruction of *A. dipsaci* on Onion Seed.

In the writer's earlier paper (1943, p. 27) it was pointed out that as even very careful winnowing and cleaning of onion seed does not get rid of the parasite, measures would have to be devised for seed treatment so as to eliminate the parasite without injury to the seed.

Warm Water Treatment.—It was shown by Cobb (1929) in the case of red clover and lucerne seed, samples of which he had found carrying the stem eelworm in a viable condition, that by soaking the seed in warm water at 118°F. for about 15 minutes the parasite was destroyed but the seed was uninjured. It was necessary to dry such seed rapidly after treatment.

The writer carried out a few tests in which infected onion seed was soaked in water maintained at 118°F. for 30 minutes, but the parasites were not killed. Further tests were made in which samples of infected seed were soaked in water at 120° to 122°F. for 30 minutes and for 1 hour. After the 30-minute treatment some of the worms resumed motility after remaining for a few days in a shallow drop of distilled water: none survived treatment for 1 hour. There are certain obvious disadvantages attaching to any method of treatment which involves the wetting and subsequent drying of seed. There must be a suitable thermostatically controlled water bath in which the seed is soaked and, in addition, it is necessary to have adequate equipment for the rapid drying of the seed after treatment. For these reasons a method was sought in which treatment could be applied to the seed in a dry condition. Fumigation was the obvious method if a substance could be found which would destroy the parasite and at the same time prove harmless to the seed.

Seed Fumigation.—Among chemicals whose properties as fumigants have been tested in recent years, one of the most efficient is methyl bromide. Its insecticidal powers were first discovered by Le Goupil (1932), who used it for the destruction of the grain weevil *Calandra granaria*. Since that time it has been extensively tested, particularly in the United States of America, both in the laboratory and in large scale fumigations for the control of many different kinds of insect pests. Mackie (1938) showed that 6 years after its use as an insecticide had been discovered its efficiency had been proved against insect pests of fruit and vegetables, herbaceous plants and woody perennials, and that it was being widely employed for the destruction of insects in stored products such as grain and dried fruits. He quoted results obtained at the California State Seed Testing Laboratory showing that it had no injurious effect on the germination of seeds of barley, wheat, peanut, carrot, bean, turnip and lettuce. Fisk and Shepard (1938) also showed that it did not deleteriously effect the germination of maize, oats, barley, wheat, beans and marrowfat peas, though Francolini (1935) had found that at dosages of 55 mgs./l applied for 24 hours there had been a very slight depression in the germination of wheat, barley and oats. The literature dealing with methyl bromide and its application as an insecticide is already quite extensive and many references to relevant papers are given in a recent paper by Richardson, Johnson *et al.* (1943). It has also been found to be a very efficient nematocide by workers in the United States for the destruction of the root-knot nematode, *Heterodera marioni,* the root-lesion nematode, *Anguillulina pratensis*, and the infective larvae of certain nematode parasites of mammals. [See Taylor and McBeth (1940) and (1941), Gingrich and Haensler (1941), Godfrey and Young (1943), Swanson and Taylor (1943) and Andrews, Taylor and Swanson (1943).]

Methyl bromide is a liquid at temperatures below 4·5°C. above which it is a gas. It possesses a number of properties which make it very suitable for use as a fumigant. It is highly toxic to insects and mites and though quite toxic to man, is not dangerous to handle provided suitable precautions against inhaling it are observed. It is not inflammable and does not produce explosive mixtures with air. It has a high vapour pressure and great powers of penetration. Residual traces of it are rapidly dispersed after treatment by bringing the substances treated into the open air. For these reasons it was decided to test methyl bromide as a fumigant for the eelworm attached to onion seed and in onion flower-part débris. The tests were so conducted

that it was possible at one and the same time to determine its lethality to the parasite and any effect which it might have on the vitality of the seeds.

Methods of Seed Fumigation.

Test Bottle.—A convenient piece of apparatus in which to carry out small scale experiments was made by fitting up a test bottle in the following manner. Wide-necked glass bottles, such as are used for the storage

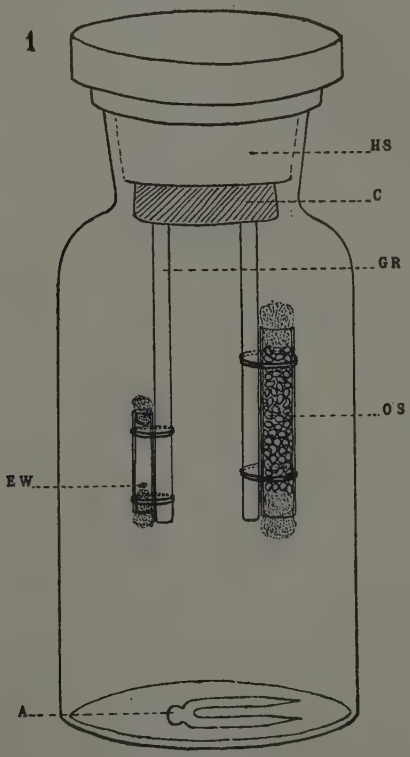

Fig. 1. Line drawing of test bottle. A, ampoule, C, cork, EW, eelworm "wool," GR, glass rods, HS, hollow stopper, OS, onion seed.

of dry chemicals were obtained, each having a capacity of a little over 800 c.c. Each bottle was provided with a well-fitting, ground-in, hollow glass stopper and in the hollow a good solid cork was tightly fitted (fig. 1). About 1 cm. on either side of the centre of the cork two

holes were bored and in each a length of glass rod was inserted having a thickness of 4·5 mm. and a length such that 9 cm. remained outstanding from the cork face. The exposed surface of the cork was then given a good dressing of cellulose dissolved in amyl acetate and, when this was dry, a further coating of a quick-drying cellulose paint so as to render the cork impervious to gas and thus prevent any leakage of the fumigant into the cavity of the stopper above the cork. To each rod was attached, by means of strong thread, a piece of glass tubing, closed at either end with a plug of cotton wool, to receive the test material. One piece of tubing had a bore of 5 mm. and a length of 3 cm., the other was 7 mm. in bore and had a length of 5·5 cm. The smaller tube was used to accommodate small pieces of eelworm " wool " which had been collected from diseased onion bulbs. This material consisted mainly of many thousands of pre-adult infective larvae of *A. dipsaci* coiled watchspring-wise and closely packed together. A small piece of it no larger than a pin's head may contain from 500 to 600 worms. Dry eelworm " wool " was chosen as a convenient form in which to submit the parasite to fumigation as it was assumed that if it could be killed in this condition it would probably be killed on the seed also. The larger tube had a capacity sufficient to accommodate 200 onion seeds. The cubic capacity of the bottle when fitted up was 800 cc.

Methyl bromide, as sold by laboratory supply houses in England at the present time, is not put up in suitably small quantities for use in the test bottle just described; ampoules containing 25 gms. being the smallest quantity obtainable. The writer was fortunate, however, in getting into touch with Dr. A. B. P. Page, of Prof. J. W. Monro's Department of Zoology and Applied Entomology, Imperial College of Science and Technology, South Kensington. Dr. Page kindly arranged to make a series of glass ampoules each containing a suitable small quantity of methyl bromide.

Method of Use.—An ampoule, containing a known weight in milligrams of methyl bromide, was carefully placed in the bottle; the latter being held in a horizontal position while the ampoule was put inside. The stopper, with the attached rods carrying the charged tubes, was then inserted and made tight after being given a thin coating of glycerine so as to form a gas-tight seal. By a vigorous shake the ampoule was broken in the bottle, which was then placed in the incubator at 24°C. and left there for the duration of the test. At the end of each experiment the stopper was removed from the bottle, the eelworm " wool " was placed in a shallow glass well with a small

quantity of distilled water and left for the worms to revive or not. Any living specimens resumed motility but those that had been killed, although absorbing water and straightening out, did not become motile. The seeds were placed on damp filter paper in Petri dishes, each containing 100 seeds, and left to germinate in the incubator at 24°C. It was thus easy, by daily counts, to determine the percentage germination of any given sample exposed to treatment. When it became necessary to determine the efficacy of the fumigant against the parasite contained in dry flower-part fragments, such as may occur amongst onion seed, small quantities of such fragments, known to be infected, were enclosed in a muslin bag which was tied to one of the glass rods carrying a tube. These fragments, after treatment, were soaked in sterile distilled water and any contained worms were teased out and examined for signs of motility. In order to provide conditions suitable for such worms to manifest motility they were, in all cases, removed from the dish in which the flower-part débris had been soaked and transferred to clean distilled water in shallow glass wells as under such conditions they are more likely to revive than in the presence of bacteria and any products of organic decay. They remained in clean distilled water for 7 to 21 days and if, by the end of this time, no motility was exhibited the worms were considered to have been killed by the fumigant.

In the following account concentrations of methyl bromide are given as milligrams per litre which, incidentally, is practically equivalent to ounces per 1,000 cubic feet. Figures for these concentrations were easily determined since each test of the fumigant was made by breaking an ampoule, containing a known weight of the chemical in milligrams, in a bottle of 800 c.c. capacity. Thus an ampoule containing 32 mgs. of methyl bromide in 800 c.c. represents a concentration of $\frac{32 \times 1,000}{800} = 40$ mgs. per litre.

It was shown by Hamilton (1941) that the requirement for a complete kill of the common red spider, *Tetranychus telarius* (L.), with methyl bromide was essentially a product of the concentration and duration of exposure in hours at any given temperature. English (1943) working on scale insects of camellias and azaleas found the same relationship to hold. In the present work it has been found convenient to express dosage by a figure representing the product of the concentration in milligrams per litre (c) and the duration of exposure in hours (t);

all tests being conducted at 24°C. Thus a dosage of 1,000 may be arrived at in a number of ways, e.g. by a high concentration of, say, 250 mgs. per litre for 4 hours or by a much lower concentration for a longer time, say 50 mgs. per litre for 20 hours.

Killing of Eelworm "Wool."—As no experimental data were available to indicate the probable toxicity of methyl bromide to dry, infective larvae of *A. dipsaci* such as occur in eelworm "wool" or attached to onion seed or in onion flower-part débris, a first few tests were made in which comparatively high dosages were given.

Table I.

Exp. No.	C=mgs./l.	T=hours	Dosage=C×T	Result
1	103	24	2,472	All worms killed
2	55·2	24	1,325	,, ,, ,,
3	29·4	24	705	,, ,, ,,
4	60·6	6	364	About 30–40% alive
5	119	4	476	,, 10% alive
6	311	2	622	,, 20% alive
7	46·5	10	465	All worms killed

It was clear from tests 1, 2 and 3 that methyl bromide effectively destroyed the parasite in the form of eelworm "wool" when applied for 24 hours, and further tests were then made in order to determine the minimum lethal dose and the effect of varying the duration of exposure to the fumigant. Experiments 4 to 7 helped to elucidate these points as they showed that the duration of exposure was important, that an efficient kill was not obtained by giving a comparatively high dose for a short time (exp. 6) and that, whereas at a dosage of 476, having a duration of just under 4 hours, the worms were not all killed, yet at a rather lower dosage of 465, applied for 10 hours, there was a complete kill. Exposures of 10 to 12 hours' duration are inconvenient to arrange and since, as will be shown later, the fumigant has practically no deleterious effect on onion seed, subsequent tests were made by leaving the test bottle in the incubator overnight, i.e. for 18 to 20 hours or longer.

Though from these results it was evident that at a dosage between 360 and 496, with an exposure in the region of 20 hours, the worms in eelworm "wool" are completely destroyed, it was obviously desirable to arrive at a dosage figure at which any examples of the parasite contained in flower-part débris were also killed since such fragments are apt to occur in comparatively well cleaned onion seed.

In order, therefore, to test the efficiency of the fumigant under these conditions, dry fragments from flower heads known to harbour the parasite were included in the tests as shown in experiments 8, 9 and 10, Table II. It can be seen that a dosage of 360 involving an exposure of 21 hours which effects a 95% kill of the eelworm " wool," does not completely destroy the worms in flower-part débris since a few of these showed slight bending movement several days after the completion of the test. In experiment 9 a dosage of 496, with an exposure of 18·5 hours, effected a complete kill of the eelworm " wool " but still did not completely destroy the worms in the débris, as one or two of these exhibited slight bending of the body 13 days later. None of these worms in tests 8 and 9 ever became actively motile and they all subsequently died. Experiment 10 with a dosage of 590 and an exposure of 20 hours gave complete destruction of the parasite in the form of eelworm " wool " and also in the flower-part fragments. It is concluded from this that a dosage of 600 involving an exposure of 18 to 24 hours, at 24°C. is essential for the effective destruction of the parasite on the seed and in such flower-part fragments as may happen to be mixed with it.

TABLE II.

Exp. No.	C=mgs./l.	T=hours	Dosage= C×T	" Wool "	Worms in flower débris
8	17·13	21	360	95% kill	1 or 2 bending slightly after 16 days.
9	26·8	18·5	496	all dead	1 or 2 bending slightly after 13 days.
10	29·5	20	590	all dead	All worms dead.

It must be pointed out, however, that this dosage figure of 600 should be considered as a minimum one suited to the somewhat ideal conditions of the test bottle and that for the treatment of bulk quantities of seed in a large fumigation chamber a higher dosage would doubtless be employed so as to cover various practical contingencies, such as minute leakages and the possible absorption of the fumigant by large quantities of seed or by other materials in the chamber. There would, however, be no risk of injury to the seed by using dosages up to 4 times the minimum since, as is shown below, germination of onion seed is not adversely affected by dosages of well over 2,000 involving an exposure of 24 hours.

Effect of Fumigation on Seed Germination.—In the first three experiments in which dosages of 2,472, 1,325 and 705 respectively were employed, onion seed was used which, unfortunately, had been damaged in some way during the drying following harvesting, with the result that, in the untreated state, it gave a germination rate of lower than 50%. Fortunately, however, another sample of infected seed was available which, in the untreated condition, gave a germination of 96%, and samples of this seed were used in all subsequent tests.

There is no need to set out in detail all the results of the germination tests since it was found that methyl bromide had no deleterious effect on the vitality of the seed when employed at concentrations and for times amply sufficient to destroy the parasite completely on the seed and in flower-part débris. The following results are cited :—

TABLE III.

C=mgs./l	T=hours	Dosage=C×T	Germination
46·5	10	465	92%
29·5	20	590	92%
34	20·5	700	95%
45	21	945	92%
92·6	24	2222	94%
159	90	14310	69%

Dosages of 700, 945 and 2,222, all much higher than necessary for the complete destruction of the parasite on seed and in flower-part débris, do not reduce the vitality of the seed since the germinations at these dosages are practically as good as with untreated seed. They show, too, that we have a wide upper limit below which it is possible to carry out the fumigation of seed with perfect safety. In general it was found that, as compared with untreated seed, there was a slight retardation of the rate of germination during the first 10 days or so after which sprouting of the seeds proceeded steadily for a further 20 days. Even at the extremely high dosage of 14,310, involving an exposure of 90 hours (a test which was carried out in order to determine whether the seed would finally be killed by prolonged exposure at a high concentration) there was still a germination of 69%, which was, however, rather slow and gradual. In general the results show that methyl bromide can be employed with safety over a wide range of dosages much higher than are required for the complete destruction of the parasite on seed and in flower-part fragments.

Larger Scale Tests.—Fumigation with methyl bromide of bulk samples of onion seed, 1 to 2 cwt. at a time, should prove comparatively easy since various kinds of fumigation chambers and boxes are already in use for the treatment of grain and other stored products. Meanwhile a brief account may be given of two larger-scale methods which the writer has employed with success.

1. *Tin Canister Fumigation Box.*—A large rectangular canister was cleaned inside and painted with two coats of a quick-drying cellulose paint so as to cover any exposed metal surfaces. A circular lid of the " press-in " type fitted tightly into the top whose sides measured 21·5 cm. The height of the tin was 31·5 cm. and its cubic capacity 14·876 litres. Eight pounds of onion seed was fumigated in this container. Previous examination had shown that this seed carried a light infection of the parasite and as it was required for an experiment on the incidence of neck-rot in the harvested bulbs, the opportunity was taken of fumigating it so as to kill the eelworms. The seed was put into two canvas bags each containing 4 lbs., and these were treated separately. In each case a dosage in the region of 600 was given by selecting an ampoule containing a suitable weight of methyl bromide coupled with an appropriate exposure; in one case 25 hours and in the other 29 hours. Having put the bag of seed into the canister, the ampoule, suspended on a cotton thread, was introduced and slung in position by fixing the lid tightly on to the thread. The lid was then made gas-tight by painting a layer of hot glue round the flange. When the glue was set the canister was given a vigorous shake so as to break the ampoule and was then placed in an incubator at 24°C. for the duration of the treatment. After treatment 4 to 5 gms. of the seed was removed from each bag and soaked overnight in sterile distilled water in a large Petri dish. Examination of the washings failed to reveal any living worms and it was concluded that the fumigation had proved successful.

2. *Sewer-Pipe Fumigation Chamber.*—Mention may also be made of a fumigation chamber which was made out of a large sewer-pipe. Details as to its construction need not be given, but as completed it was sunk in a concrete base, was fitted with a gas-tight lid and heated by electric lamps in a thermostatically controlled circuit. The ampoule of methyl bromide was fastened to a movable arm attached to the underside of the lid. It was broken at the appropriate time by contact with the wall of the pipe as the arm to which it was attached was

allowed to drop, by means of an electrically controlled device, whereby the wire holding the arm up was fused. The chamber was capable of holding a small sack containing 30 lbs. of seed. As this quantity of onion seed was not available, buckwheat seed was used instead and a 1 oz. sample of onion seed in a muslin bag was placed at the centre of the sack. Treatment was carried out at 75°F., which is practically the same as 24°C., at which the test-bottle treatments were done. After treatment a 4 to 5 gm. sample of the seed was taken and soaked overnight in distilled water. The washings were concentrated by centrifuging and any worms present were picked up and transferred to glass wells containing a shallow drop of water. Subsequent examination for a further 7 to 14 days or so revealed whether the worms showed any signs of bending or other movements.

Table IV.

No.	C mgs./l	T hours	Dosage C×T	Result
1	32·39	20	655·8	Incomplete kill, several showing bending.
2	38·88	26·3	1023·8	Incomplete kill, a few showing bending but all dead after 11 days.
3	58·76	27	1586·6	Complete kill.

The results of three tests carried out with this chamber are set out in Table IV. It is at once apparent that the chamber was not as efficient as the test-bottle or the tin canister in that at dosages of 655·8 and 1023·8 the kill was not complete though such worms as showed bending movements never became actively motile and they all eventually died. The third treatment at a dosage of 1586·6 was completely effective.

Discussion.

The present state of our knowledge of the occurrence of *Anguillulina dipsaci* on onion seed is now fairly detailed and the discovery of a successful method of seed fumigation for its control enables one to make certain recommendations. Though the source of the eelworm infection which onion seed may carry has not as yet been conclusively demonstrated experimentally, there is, at the present time sound circumstantial evidence which points to its arising from very lightly infected bulbs which are planted for seed production. Evidence for this has already been given in the writer's earlier paper (1943, p. 26). A second similar case has come to the writer's knowledge. In this

instance onion bulbs raised in Bedfordshire in 1942 were subsequently grown in 1943 for seed in Worcestershire at more than one centre. Seed from at least two of these crops was sent to the writer for examination and was found to be contaminated with the parasite. It seems fairly certain, therefore, that it is from the parent bulb that infestation of the inflorescence arises. It is hoped to settle the matter conclusively during the present season by planting onion bulbs for seed production obtained from three difference sources (i) bulbs known to be lightly infected and showing mild symptoms of attack; (ii) bulbs healthy in appearance but possibly carrying an infection; (iii) healthy bulbs planted in soil inoculated with the parasite.

The very lightly infected bulbs which it is believed give rise to contaminated seed are no doubt planted in the belief that they are quite healthy. Added to this is the further fact that the plants growing from them may show no outward signs or symptoms to suggest that they may be carrying a dangerous parasite. Yet when the seed is finally threshed and cleaned it may carry the parasite in numbers sufficient to give rise to disease in the resulting crop whilst still in the ground or in the bulbs during storage, as shown by Wallace and Wood (1943).

Such seed-borne infection is not confined merely to one or two onion varieties but has been found by the writer, during 1943 and 1944, to occur on at least 9 different varieties drawn from commercial samples of seed as offered for sale to the general public. Such seed is being distributed not by one or two wholesale merchants only but by several of them; a state of affairs which is, of course, unavoidable at the present time since they are quite unaware that some of their stocks of seed are contaminated with the parasite.

Apart from the careful examination of samples of all stocks of onion seed raised in this country, there is no laboratory test which can be applied to determine how widespread is the occurrence of seed-borne infection of the eelworm on onion seed. Under these circumstances the writer suggests, as a general measure of control, the fumigation of all British-grown onion seed each year after it has been threshed and cleaned. If this course were adopted over a period of years one important source of infection would be effectively eliminated and onion crops would be largely safeguarded from losses due to "bloat." The dispersal of the parasite to fresh uninfected sites would also be effectively checked.

It is not claimed that seed fumigation with methyl bromide would completely check all eelworm disease in onions in this country since there are certain areas where it is so well established that it is difficult to raise a healthy crop. Other sources of infection may also occur such as fields or plots where eelworm-infected narcissi or parsnips have been grown or where the parasite is present as an undetected infestation in some weed or other, such as chickweed or scarlet pimpernel. Seed fumigation would, however, do much to prevent the spread of the disease to previously clean sites, and if it could go hand in hand with more extensive weed destruction in onion crops and the practice of crop rotation (at present insufficiently adopted in the case of onion growing) there is no doubt that eelworm disease of onions might within a few years become much less common that it is at present.

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Summary.

1. Further investigations on the occurrence of *Anguillulina dipsaci* on onion seed are presented. It is shown by the examination of 2,000 seeds individually that the parasite is usually located on the seed in the region of the hilum. A given seed may have as many as 47 worms or only 1 worm attached to it.

2. A method of seed fumigation with methyl bromide is described whereby the parasites attached to seeds are killed but the seed is unharmed. By the use of a test-bottle technique it has been found that a minimum dosage of 600, i.e. a concentration-time product of 600 involving an exposure to the fumigant of 18 to 24 hours at 24°C. is necessary for the destruction of the parasites attached to the seeds and in such flower-part débris as may be mixed with it.

3. The fumigant has no harmful effect on the vitality of the seed at dosages sufficient to destroy the parasite nor at much higher dosages. There is thus a wide upper margin of safety in its use. Two practical methods for the fumigation of large quantities of seed are described.

4. The probable origin of seed-borne infection from lightly infected parent bulbs is discussed. It is shown that at the present time seed-borne infection is fairly widespread and has been found to occur on at least 9 different varieties of onion seed as distributed by seed merchants. Recommendations as to control are made which it is suggested would go far to reduce the incidence of the disease in this country.

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Further observations on *Anguillulina dipsaci* infestation of the onion scape and inflorescence.

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In a recent paper the writer (Goodey, 1943) has shown that the stem eelworm, *Anguillulina dipsaci*, occurred in considerable numbers in the scape, i.e. the flower stem, and the inflorescence of onions grown for seed in Sussex during 1943. The worms, mainly consisting of infective larvae, occurred in the dry papery tissues lining the hollow scape, in the pedicels of the flowers and in various of the floral organs, such as withered sepals and petals, in the receptacle and placenta and on the walls of the seed capsule. They were also attached to the outside of the ripened seed. In a second paper (Goodey, 1945) it is further shown that the parasite becomes firmly attached to the seed chiefly in the region of the hilum, whence it cannot be removed by the usual processes of winnowing and cleaning. The foregoing results were obtained from plant material in a dry condition and seed samples which had been threshed and cleaned. Study of this material had made it evident that the parasite must somehow gain access to the living tissues of the scape and inflorescence as these grow from the parent bulb, but it was not apparent how this came about; whether from lightly infected parent bulbs or direct from the soil. Circumstantial evidence had been adduced in the two papers, already mentioned, pointing to lightly infected parent bulbs being the probable source of infection, but the whole matter needed further investigation to elucidate this and other points. In the present communication the results of studies directed to the solution of these questions are given.

Onion Seed Production.

The onion is a biennial plant and its seed is formed in the flowers produced in the second year of its growth. In practice ripened bulbs are usually planted in the autumn or in the spring of the following year. During the course of the season first leaves and then one or more scapes are produced by each bulb. Each scape bears at its top an umbel-like inflorescence consisting of numerous flowers each supported on a slender pedicel. Each individual flower is made up of a perianth consisting of 6 equal elements; 3 sepals and 3 petals which are whitish or pale green in colour. Within the perianth are 6 stamens

and at the centre of the flower is the pistil consisting of a knob-like ovary crowned with a simple style. The ovary is made up of 3 carpels each of which accommodates 2 ovules. After fertilization has taken place the tissues of the ovary grow rapidly whilst the perianth and the stamens shrink and ultimately become dry and scaly. Finally, towards the end of summer or in early autumn the scape and the inflorescence begin to dry out and the seed capsule splits down the side walls revealing the black seeds within.

One matter which was very obscure and required explanation was how it was possible for the parasite to be transmitted to seed in the second season when it is often so destructive to the bulb in the first season as to destroy it. At first sight it seemed almost impossible that so damaging a parasite could be harboured by an onion bulb without completely destroying it, and yet clear evidence was available, from the dry material studied in 1943, that the parasite could occur in large numbers throughout the length of the scape and in the inflorescence. As a satisfactory working hypothesis to account for such an occurrence it had to be supposed that there must be bulbs which carry the parasite in such small numbers as not to be seriously affected by them, and that such apparently sound bulbs, when planted for seed production, give rise to infected scapes and inflorecences.

Material.

In order to test the matter and to trace, if possible, the course taken by the parasite in reaching the flowers, three separate sources of material were used as follows :—(1) Onion bulbs known to be lightly infected. (2) Onion bulbs possibly lightly infected. (3) Healthy onion bulbs planted in infected soil.

Onion Bulbs Known to be Lightly Infected.—In the autumn of 1943 18 onion bulbs of the variety Up-to-Date were sent to the writer by Mr. E. R. Wallace, of the Agricultural Institute, Kirton, Boston, Lincs. These were from a crop in which a number of bulbs lightly infected with the eelworm had been found after they had been lifted and carefully inspected for the presence of neck rot. (See Wallace & Wood, 1943.) Each of the 18 bulbs was only mildly affected; one or two showing a scale broken at the base, another having a small longitudinal crack or perhaps a bulb had a somewhat puffy feel over a dead-white area : all symptoms described by Wallace & Wood in their paper. The bulbs were planted on November 8th on an experimental plot which had not previously carried onions. During the

winter some of them showed signs of growth, but as spring advanced they all became more and more seriously diseased and finally died out.

In April, 1944, a further batch of 18 bulbs of the same variety and from the same crop were received from Mr. Wallace, who reported that when examined in store during January and February each bulb was apparently quite firm and sound and would have been passed as healthy. By early April all 18 were showing mild symptoms of infection with eelworm similar to those described above. These bulbs were planted in a garden plot on April 17th where onions had not been grown before. Signs of growth soon made their appearance in all of them as a tuft of leaves, but in one after another a gradual rot took place so that, finally, 17 of them died and only one was left which produced two scapes and inflorescences. These had a perfectly healthy appearance, the scapes remained green and each inflorescence was almost spherical in shape and had practically no abortive flowers. When examined for the presence of the parasite one scape and inflorescence was found to be infected abundantly whilst the other was quite free from nematodes. From these findings it is concluded that bulbs carrying a light but patent infection mostly succumb to disease when grown on. It is improbable, therefore, that it is from such bulbs that the infection of the scape and inflorescence commonly arises.

Onion Bulbs Possibly Lightly Infected.—In November, 1943, Mr. E. G. Thompson, of the National Institute of Agricultural Botany, Cambridge, sent the writer a small sackful of ripened onion bulbs. These belonged to various varieties which had been grown on the trial grounds there during 1943 on land which had never been previously cropped with onions, but where oats had been grown during the preceding 5 or 6 years and had suffered from " tulip-root " caused by *A. dipsaci*. During the growing season considerable areas of these onions had suffered severely from eelworm " bloat," and when the writer visited the plot in September, 1943, there were a number of bare patches where the bulbs had been completely destroyed. Around these patches were many well ripened and apparently healthy bulbs. The sackful of bulbs sent by Mr. Thompson had been collected from around the bare patches and it was hoped that some, at least, of them would be carrying very mild infections such as might give rise to infected scapes and inflorescences. All the bulbs were quite firm and sound on arrival and none of them showed even the mildest symptoms of eelworm attack such as a cracked scale or broken base or white,

puffy areas. After being spread out on a bench for a week or so they were all planted early in December on a small plot of ground where onions had not been grown before. There were 8 rows each containing 14 to 16 bulbs. Some of them failed to grow and rotted, but as spring advanced about 100 plants had become established and were sending up scapes. In due course most of them grew well and had to be tied up to stakes for support. Some of the bulbs produced only one scape, but many others sent up 2, 3 or 4 and a few 6 or 7.

Examination of scapes for the presence of eelworms was begun about the middle of June, when it was found that though some of them were free from the parasite others contained it in the central tissues. Later examinations revealed the parasites at the top of the scape and entering the inflorescence; details, however, of the upward progress of the worms are given later on. In September, 1944, the scapes and seed heads were harvested by cutting the scape at the base. The produce from each bulb was labelled separately, and the stems with their attached heads were hung up to dry for about a week in an airy shed. Subsequently each scape was examined for the presence of the parasite in the innermost tissues just below the origin of the inflorescence and at one or two points throughout its length. In this way the number of infected scapes, and thus of infected bulbs, was determined. Of the produce of 98 bulbs examined in this way, 18 were found to be infected with *A. dipsaci*, i.e. 18·4%. These 98 bulbs had given rise to 335 scapes of which 27 were infected, i.e. 8·05%. In the case of those bulbs with more than one scape there was considerable variation in the range of their infection. In some cases one out of two was infected, in others one out of three and in others one out of four. In another, two out of four, whilst in one instance all four, and in another all six were infected.

These observations clearly indicate that *A. dipsaci* can be carried by apparently healthy onion bulbs which exhibit no recognisable signs of the presence of the parasite within, and the conclusion seems warranted that it is probably such bulbs which give rise to infected scapes and inflorescences and so, ultimately, to seed contaminated with the parasite.

Healthy Onion Bulbs Planted in Infected Soil.—In order to test whether perfectly sound onions could become infected and produce parasitized flowers when planted in infected soil the following procedure was adopted. In a small plot of ground, 3 ft. long by 1½ ft. wide,

two drills about 3 ins. deep were drawn, and in each was distributed a liberal dressing of small dry fragments of onion scape known to be heavily infected with *A. dipsaci*. This infective material was obtained from the scapes sent by Mr. S. G. Jary, which had been grown near Chichester and had been found to contain many thousands of the parasite. (See Goodey, 1943.) The inoculum was covered with about 2 ins. of soil and made firm, and then 9 healthy onion bulbs, of the variety Bedfordshire Champion, were planted by pressing into the soil of the rows. These bulbs were known to be free from infection as they had been grown on a plot in the writer's home garden where "bloat" had never occurred. Planting was done on November 9th, 1943. Early in 1944 leaves appeared from all 9 bulbs and by June all of them had sent up scapes which had a fairly healthy appearance. One or two of the scapes began to show rather premature yellowing in their lower regions, and on June 12th one of them which had become weakened at the base and had fallen over, was brought into the laboratory and examined for the presence of the parasite. Successive cuts were made across the scape at 1 in. intervals from the base upwards and small portions of the innermost tissues were teased out in water and examined under the microscope. Adults, eggs and larvae of *A. dipsaci* were found to be present as far as 15 ins. from the base. Later on, at various times during June and July, other scapes from these bulbs were examined in the same way and in due course the worms were found to have reached the top of the scape and to have invaded the inflorescence.

These findings made it evident that if perfectly healthy onion bulbs are planted in infected soil the resulting scapes and inflorescences may become infected and so yield seed contaminated with the parasite. These results were entirely unexpected by the writer who had tacitly assumed that the tissues of a well ripened bulb would probably not become parasitized. The observations recorded above show, however, that this was quite an erroneous assumption, and it is now clear that *A. dipsaci* can enter a healthy bulb. It probably does so *via* the fractures at the base of the bulb scales caused by the emergence of the roots.

Bringing together the results of the three foregoing trials, the following conclusions may be drawn. (*a*) Recognisably diseased onion bulbs, even though but mildly affected, generally succumb to disease before they succeed in producing the scape; one only out of 36 such

bulbs succeeded in growing and producing a flower-bearing scape. It is improbable, therefore, that it is from bulbs showing patent symptoms of attack that the cryptic infestation of the scape and inflorescence arises. In any case even a lightly affected bulb, especially if at all soft, would probably be rejected as suspect by any sensible grower. (*b*) Onion bulbs healthy in appearance and quite firm to the touch may give rise to apparently normal scapes and flowers. One or more of the scapes, or perhaps all of them, from a bulb with such a latent infection may become parasitized and thus give rise to an inflorescence containing large numbers of the parasite and so to contaminated seed. It seems probable that it is commonly by this means that the parasite becomes seed-borne. It is a common practice for seed merchants to get farmers and nurserymen to grow onion seed under contract from bulbs supplied by the merchants. If among the bulbs there are some with a latent infection the resulting crop is certain to yield seed contaminated with the parasite, and it can be easily understood how, by this means, the parasite may become widely distributed through commercial channels to nurserymen and to the general gardening public. (*c*) Though possibly unlikely to happen often in commercial practice, the results of the third trial show that if bulbs, known to be free from the parasite, happen to get planted in soil infected with the parasite, the resulting crop may yield contaminated seed. Whether at the present time, this is an actual source of infected seed is unknown. It seems to the writer, however, that it is likely to occur now and then on allotments and small holdings, especially in the Bedfordshire area, where the parasite is very widespread and where growers commonly raise their own seed.

Two recommendations at least can be made. (1) Avoid using onion bulbs for seed production if they have been grown where onion "bloat" has occurred as it is probable that some of them, though quite healthy in appearance, will contain the parasite and yield contaminated seed. (2) Avoid planting healthy bulbs for a seed crop in soil known to be infected with the onion eelworm as the resulting flowers may become parasitized and yield contaminated seed.

Invasion of Scape and Inflorescence.

Before dealing with the progress of the parasite up the scape and into the flowers, a brief account may be given of the structure of the scape itself. The full-grown scape may attain a length of 2 to 3½ ft. It is slightly bulbous below where it is surrounded by the remains of

the parent bulb. It then narrows somewhat in the neck region formed by the closely investing leaf-bases and is solid in structure with a central white core composed of large parenchyma cells. Proceeding upwards it begins to expand in width and forms the characteristic swelling which is situated below the middle of its length. When cut across 2 or 3 ins. below the swelling it will be found that the scape is now hollow inside and that the central parenchyma has spread out to form the inner lining. Just within the lining is a series of straight vascular bundles which extend from the base to the top of the structure. Outside these is a fairly rigid cortex covered externally by the epidermis. Above the swelling the scape tapers considerably until at its top it is about $\frac{1}{4}$ to $\frac{1}{2}$ in. wide. Here the hollow tube is closed by a layer of very vascular tissue and the inner parenchyma again fills up the central cavity in its topmost half inch or so.

Examination of scapes for the presence of the parasite was begun, as already mentioned, on June 12th, when one of them growing from a healthy bulb planted in inoculated soil was found to be infected. In this and later examinations the procedure adopted was generally as follows. A small portion of the solid tissue from the centre of the extreme base of a scape was dug out on the point of a scalpel, placed in distilled water in a solid glass well and teased out with needles. At the same time note was taken of the colour of the tissues, whether quite white or discoloured cream or buff. If the parasite was not found in this situation and especially if the tissues were quite white it was generally completely absent throughout. The scape was then cut across 1 in. from the base and a further small portion of the central tissues taken out and examined after teasing up under the microscope. The process was then repeated inch by inch, or occasionally at 2 in. intervals, up the length of the scape, and in this way evidence was obtained of the distance which the worms had travelled upwards. In the first scape they had reached 15 ins.; in the second (taken from the same plant) they were 10 ins. from the base; in a third example taken from another and measuring 3 ft. long, they were 16 ins. up at the level of the swelling. In all cases adults, eggs and larvae in very variable numbers were present amongst the large parenchyma cells.

On June 23rd a scape 25 ins. long, growing from a bulb sent by Mr. Thompson, was taken; it was one of four scapes produced by the same bulb. In this case examples of the parasite were found at 2 in. intervals up to 22 ins. from the base. The scape was then cut through

at about ½ in. below the origin of the inflorescence and a few fairly thick median longitudinal sections were cut by hand. On placing these in water, a few adults of the parasite floated out from the central parenchyma cells. The sections were then treated with boiling 80% acetone, following Godfrey's (1935) method for the preparation of shoot material which is to be processed with Flemming's solution for the demonstration of nematodes. After washing for two to three hours the sections were fixed in strong Flemming's solution for 20 to 30 minutes. They were then washed overnight in running water, followed by passage through graded alcohols up to absolute alcohol. After clearing in oil of cloves they were mounted in Canada balsam. Examination of the sections showed many eggs and one or two larvae of the parasite in the central parenchyma close to the end of the scape. Pedicels and flower rudiments had not yet been invaded. The foregoing technique was adopted in the handling of all subsequent hand sections of the top of the scape and the inflorescence.

It is unnecessary to give details of the several scapes which were examined from now on, at intervals of a week or 10 days, throughout July and the first two weeks of August, since the picture presented in each case had a common pattern. Adults, eggs and larvae of the parasite, sometimes in large numbers (see fig. 1) were found in the inner parenchyma of the scape. They occurred also in large numbers in the tissues just below the inflorescence (fig 2), and were now found to be making their way up the individual pedicels (fig. 3) and into the various floral organs. Many scapes were found which were becoming pale yellow in patches from below upwards. In these cases the central tissues at the extreme base were generally more or less discoloured cream or buff, and the inner parenchyma cells were often soft and mushy. This condition was apparently due to the presence of secondary invading bacteria which attacked the cells. On such scapes the inflorescence might be composed entirely of flowers which had failed to develop; in other cases there might be normal flowers as well as aborted ones. The yellow discolouration of the scape and the failure of flowers to develop seems to be the result of the action on the tissues of these secondary invaders rather than that of the eelworm alone since one or two scapes were found which remained quite green, and although heavily invaded by *A. dipsaci* the parenchyma cells were not mushy and very few aborted flowers were produced.

Where an inflorescence is composed of few or many aborted flowers the parasite may make its way into them (fig. 4), and it is clear that

when this sort of material is harvested the parasite will be present in the dry fragments of the malformed flowers.

Transverse sections of invaded pedicels, each of which has 6 fine vascular bundles, revealed the fact that the parasites, in passing through this region, break down the cells in their course and form narrow channels. Though each pedicel is very narrow and is composed of compact small-celled tissue, the worms reproduce during their passage through the organ for many adults as well as eggs and larvae were found within (fig. 7). In some cases in the expanded head of the pedicel which forms the flower receptacle, a fairly large central cavity may be formed which is filled with a mass of nematodes. Having passed through the pedicel the worms make their way, as already mentioned, into the various floral organs. In young flowers they were found in the sepals and petals (figs. 5 and 6), in the stamens, both in the filament and the anthers, in the central tissues of the placenta and in or on the ovary walls. In older flowers, examined towards the end of July or in early August, when the segments of the perianth are shrivelling up and the ovary is showing considerable enlargement, adults, eggs and larvae were found in good numbers in the receptacle and in the rather dense tissues of the placenta (fig. 8). It was clear from these flowers that the worms were now favourably situated for attachment to the seeds (the short funicles of which spring from the central mass) as these increase in size and ripen. The observations on the growing plant had, in fact, brought one to the point arrived at in the examination of dry, infected flowers twelve months earlier in August, 1943, in that they had enabled one to trace fairly completely the course followed by the parasite in reaching those tissues from which attachment to the seed could take place.

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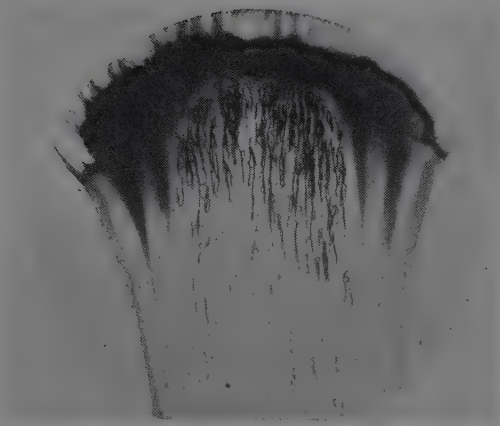

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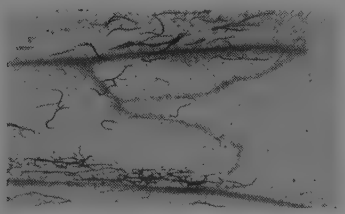

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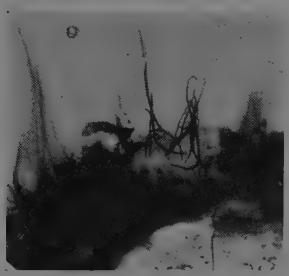

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Plate 1. Photomicrographs of portions of onion scape and inflorescence parasitized by *Anguillulina dipsaci*.

1. Small portion of inner parenchyma of scape with adults and eggs of *A. dipsaci*. ×7.
2. Portion of freehand longitudinal section of top of scape showing large numbers of the parasite in the central parenchyma; the bases of the pedicels are seen above. ×7.
3. Part of longitudinal section of top of scape showing *A. dipsaci* invading the base of two pedicels; eggs of parasite in central parenchyma of scape. ×15.
4. *A. dipsaci* within the perianth of an aborted onion flower. ×15.

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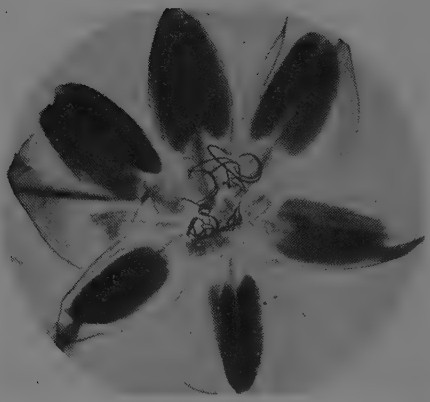

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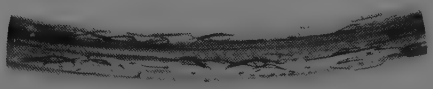

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Plate 2. Photomicrographs of portions of onion flowers showing *A. dipsaci*.

5. Portion of the perianth with attached stamens showing numerous specimens of *A. dipsaci* in sepals and petals. ×15.
6. Onion flower from which the pistil has been removed; the perianth has been inverted and spread out; *A. dipsaci* shown in the central area. ×12.
7. Portion of a pedicel of an onion flower showing adults and eggs of *A. dipsaci* in tissues. ×12.
8. Freehand longitudinal section through a developing seed capsule and top of the pedicel of an onion flower; the elements of the perianth have been removed. Adults and eggs of *A. dipsaci* located in the tissues of the receptacle, *i.e.*, the swollen head of the pedicel, and in the placenta. A collapsed developing seed with short seed stalk is seen on the left. ×13·5.

Journal of Helminthology, Vol. XXI, *Nos.* 2 *and* 3, 1945, *pp.* 69–70.

A note on the subfamily *Turbatricinae* and the genus *Turbator* Goodey, 1943.

By T. Goodey, D.Sc.

(*Institute of Agricultural Parasitology, St. Albans.*)

In a recent paper the writer (Goodey, 1943) dealt with the systematic position of the vinegar eelworm and certain related species. A new subfamily, *Turbatricinae*, was erected and a new genus, *Turbator*, proposed for 7 species possessing morphological features differentiating them from the vinegar eelworm, *Turbatrix aceti* (Müller, 1873) v. *aceti* Peters, 1927. In regard to this paper, Dr. Gerald Thorne has sent a letter to the writer in which he has been kind enough to draw attention to two points which, in his view, call for emendation and correction, as follows.

1. It appears that in establishing the subfamily *Turbatricinae* the writer failed to designate a type genus for it, although the genus *Turbatrix* was dealt with first and might, by implication, be considered as type of the new subfamily. In order that there may be no mistake in the matter *Turbatrix* Peters, 1927, is herewith formally designated as type genus of the subfamily *Turbatricinae*.

2. The name *Turbator* appears to be invalid since it is antedated by the name *Panagrellus* Thorne, 1938, a species of which, viz., *Panagrellus pycnus* Thorne, 1938, was shown to belong to the same genus as the sour-paste eelworm and 5 other species which the writer placed under *Turbator*. Since *Panagrellus pycnus* and the sour-paste eelworm undoubtedly belong to the same genus it becomes necessary, according to the Law of Priority, International Rules of Zoological Nomenclature, Article 25, to substitute the name *Panagrellus* Thorne, 1938, as the valid name of the genus in place of *Turbator* Goodey, 1943, which thus becomes a synonym of it. Dr. Thorne reports that he has found in young females of *Panagrellus pycnus* a post-vulval uterine sac, such as occurs in the other species of the genus, but which was not described in the original account of *P. pycnus* based on older specimens in which the structure was obscured. The occurrence of this sac lends additional support to the inclusion of *P. pycnus* and the sour-paste eelworm in one and the same genus.

The species of *Panagrellus* are as follows :—

Panagrellus redivivus (Linn., 1767) new comb.

SYN. *Turbator redivivus* (Linn., 1767), Goodey, 1943.
Turbatrix rediviva (Linn., 1767), Peters, 1927.
Anguillula rediviva (Linn., 1767), Stiles and Hassall, 1905.
Anguillula glutinis (Müller, 1783).
Cephalobus parasiticus Sandground, 1939.
The sour-paste eelworm.

Panagrellus ludwigii (de Man, 1910) new comb.

SYN. *Turbator ludwigii* (de Man, 1910), Goodey, 1943.
Turbatrix ludwigii (de Man, 1910), Peters, 1927.
Anguillula ludwigii de Man, 1910.
In white slime-flux of oak, Germany and England.

Panagrellus silusiae (de Man, 1913) new comb.

SYN. *Turbator silusiae* (de Man, 1913), Goodey, 1943.
Anguillula silusiae de Man, 1913.
In so-called beer felts, Germany and France.

Panagrellus nepenthicola (Menzel, 1922) new comb.

SYN. *Turbator nepenthicola* (Menzel, 1922), Goodey, 1943.
Anguillula nepenthicola Menzel, 1922.
In pitchers of pitcher plants, Dutch East Indies.

Panagrellus leucocephalus (Steiner, 1936) new comb.

SYN. *Turbator leucocephalus* (Steiner, 1936), Goodey, 1943.
Neocephalobus leucocephalus Steiner, 1936.
In an agar culture inoculated with wood from scarlet oak and apparently feeding on a fungus, U.S.A.

Panagrellus pycnus Thorne, 1938.

In a slime-flux from a Great Plains cottonwood tree, *Populus sargentii* Dode, near Magna, Utah, U.S.A.

Panagrellus redivivoides (Goodey, 1943) new comb.

SYN. *Turbator redivivoides* Goodey, 1943.

REFERENCE.

GOODEY, T., 1943.—On the systematic relationships of the vinegar eelworm, *Turbatrix aceti*, and its congeners, with a description of a new species. *J. Helminth.*, XXI (1), 1–9.

Journal of Helminthology, Vol. XXI, *Nos.* 2 *and* 3, 1945, *pp.* 71–84.

On *Heterodera cruciferae* n.sp. of Brassicas, and on a *Heterodera* Strain infecting Clover and Dock.

By Mary T. Franklin, Ph.D.
(*Institute of Agricultural Parasitology, St. Albans.*)

Before the separation from *Heterodera schachtii* of the three clearly marked species *H. rostochiensis, H. göttingiana* and *H. major,* the host range of the original *H. schachtii* included over 170 plants from 29 Families. *H. schachtii* was considered as comprising several biological strains, most of them having very definite host ranges. Certain morphological differences being later observed between some of these so-called strains, it became necessary to raise three of them to specific status (Franklin, 1940). We can now distinguish the species *H. rostochiensis* attacking potatoes, tomatoes and occasionally two or three other Solanaceous plants : *H. göttingiana* attacking garden peas, broad beans and (according to Walton, Ogilvie and Mulligan, 1933) vetches and red clover. *H. major* attacking cereals, several grasses (including *Lolium perenne* and *L. italicum*) and red clover (Goffart, 1932 and Edwards, 1935) : and *H. schachtii* noted mainly for its attacks on sugar beet, mangolds and Brassicas, but also found sporadically on a large number of other plants. All infections which are not obviously due to one of the first three species are generally considered as being caused by *H. schachtii.*

It is now possible to distinguish, amongst what may eventually prove to be a collection of species, comprised within *H. schachtii,* one which shows certain well marked differences from the true sugar beet eelworm. This species appears principally to attack Cruciferous plants, and will for the moment be called the " Brassica eelworm."

Occurrence of the Brassica Eelworm.

In the summer of 1943, during the examination of soil from a long established garden where the presence of *H. rostochiensis* had just been noticed, a considerable number of small lemon-shaped cysts was observed. These were obviously not potato eelworm cysts, and it seemed unlikely, from the history of the ground, that they were of the sugar beet race. At first it was thought that they might have originated on clover or dock plants, which have been found infected in the neighbourhood, or on some other weeds. However, an examination of

the roots of some old cabbage stumps growing on the ground where the soil samples had been taken showed cysts resembling those found in the soil. Cysts collected from these roots were placed in pots of sand or sterilised soil in which various test plants were grown from seed. As the number of cysts was not very great, soil from the infected plot was used in a further series of susceptibility trials. After some weeks' growth the roots of the experimental plants were examined for cysts, and if cysts were not found a portion of the root system was stained with lactophenol-acid fuchsin and examined for the presence of *Heterodera*. The following results were obtained :—

Infected (mature females found in all cases)—

(*a*) Grown with cysts from cabbage roots—red cabbage, white mustard, radish.

(*b*) Grown in soil from the infected plot—cabbage, broccoli, swede, brussels sprouts, white mustard, cress (*Lepidium sativum*), wallflower (*Cheiranthus cheiri*).

Not infected—

(*a*) Grown with cysts from cabbage roots—sugar beet, spinach beet, shepherd's purse (*Capsella bursa-pastoris*), *Rumex crispus, Galeopsis speciosa, Urtica dioica.*

(*b*) Grown in soil from the infected plot—sugar beet, spinach beet, garden pea, oats, wheat, *Lolium perenne, Dactylis glomerata, Agrostis alba, Trifolium pratense, T. repens, Reseda odorata,* carrot, parsley (*Petroselinum hortense*), shepherd's purse.

It was thus proved that the Brassica eelworm was not identical with that found locally on clover and dock, nor could it be the well-known sugar beet race, as in two tests neither sugar beet nor spinach beet was infected. In May, 1944, sugar beet, red beet and mangolds were sown on the infected plot at the same time as several varieties of Brassica. The roots were examined in July and again in August, both in the fresh state and after staining in lactophenol-acid fuchsin, but no *Heterodera* could be found in any of the Chenopodiaceous plants, although the Brassicas were infected.* All the test plants which became infected are members of the Cruciferae, and one, *Cheiranthus cheiri,* has not hitherto been recorded as a host of *Heterodera.*

*Acknowledgments are due to Professor R. T. Leiper for this information.

Host Ranges of the Sugar Beet and Brassica Eelworms Compared.

In order to obtain first-hand information on the host range of the sugar beet eelworm for comparison with that of the Brassica eelworm, in so far as it is known, cysts were removed from the roots of mangolds grown during 1943 on an infected experimental plot, where sugar beet and mangolds have been cultivated and have been infected for a number of years. These cysts were mixed with sand or sterile soil in which seeds of various plants were sown. A second series of plants was grown from seed in soil from the infected plot. The following results were obtained when the roots of the plants were examined some weeks later :—

Infected when grown with cysts from mangolds (cysts found in all cases)—

Sugar beet, radish, white mustard, cabbage, red cabbage, swede, *Rumex crispus, Galeopsis speciosa.*

Infected when grown in soil from the infected plot—

Red beet, spinach (*Spinacea oleracea*), broccoli, brussels sprouts, shepherd's purse, virginian stock (*Malcolmia maritima*), *Trifolium repens, T. pratense,* garden pea (lightly). There were no cysts on parsley (*Petroselinum hortense*), but when 3 or 4 seedlings were stained one *Heterodera* larva was found, at a time when the susceptible plants, grown under the same conditions, already had white cysts on the roots. A single larva was found similarly in the roots of a carrot seedling.

Not infected when grown in soil from the infected plot—

Cheiranthus cheiri and *Lepidium sativum;* the latter was reported by Goffart in 1936 as infected by the sugar beet eelworm.

It is possible that in the plot infected with the sugar beet eelworm there is more than one species of *Heterodera,* but this point will be dealt with when the dock-clover strain is being considered. The infections on carrot and parsley are doubtful. Triffitt described an infection of carrots in 1931 and then thought that the potato race might be responsible, but the cysts were lemon-shaped and more than one species of *Heterodera* may have been present in the field under observation. The very slight infections now found on carrot and parsley need further confirmation before they can be accepted.

Bionomics of the Sugar Beet and Brassica Eelworms Compared.

The reaction of the cysts from mangolds and cabbages to root excretions of their various hosts was next tested. Batches of 50 cysts from each of the two hosts were set up in Fenwick's single cyst testing apparatus (Fenwick, 1943). The batches were tested with (1) distilled water, (2) sugar beet root excretion, (3) mustard root excretion, (4) cabbage root excretion, (5) swede root excretion and (6) radish root excretion. The root excretions were obtained from seedlings of the various plants growing in sand in the case of the sugar beet, and on muslin stretched over Petri dishes of distilled water in the other cases. The solutions were renewed at weekly intervals when counts of the hatched larvae were made, and the experiment was continued for 4 weeks. By this time if there were to be any stimulation it would have occurred. Hatching was very uneven, many cysts yielding no larvae at all, although they had been carefully selected and soaked before being set up, and had been obtained from cabbage roots and from soil brushed off infected mangolds respectively. At the end of the experiment all the cysts were dissected and it was found that in many of them all or nearly all of the larvae appeared dead, remaining bent when released from the egg membrane. (This may have been due to unfavourable storage conditions.) When the mean number of larvae hatched per cyst was calculated, the number of cysts from which no larvae had hatched and in which they all appeared dead was therefore subtracted from the original 50 which had been set up. The results are set out in the following table :—

Stimulant	Mangold cysts		Cabbage cysts	
	Mean larvae/ cyst in 4 weeks	No. of cysts	Mean larvae/ cyst in 4 weeks	No. of cysts
Distilled water	34·4±16·04	16	1·19±0·418	33
Sugar beet root excretion ...	19·2± 6·47	10	1·76±0·364	30
Mustard ,, ,, ...	5·6± 2·60	5	3·55±0·828	29
Cabbage ,, ,, ...	22·6± 8·51	11	5·73±1·001	30
Radish ,, ,, ...	22·0±10·09	14	8·00±1·184	31
Swede ,, ,, ...	23·4± 9·37	16	12·80±3·947	28

If the means and standard errors are compared it will be seen that in the case of the mangold cysts no hatch differs significantly from that in distilled water, except that in mustard root excretion, which is significantly lower than any of the others. On the other hand, the

Brassica cysts in distilled water and in sugar beet root excretion gave significantly fewer larvae than those in the other root excretions, while a significantly greater number of larvae hatched in radish and in swede root excretions than in either mustard or cabbage root excretions. It must be remembered that these results are based on numbers of larvae hatched from rather small numbers of cysts, particularly in the case of the cysts from mangolds, but they indicate at least that larvae from Brassica eelworm cysts are probably not stimulated by sugar beet root excretion, which has been shown by Schmidt (1930) to stimulate hatching of the true sugar beet eelworm. The sugar beet seedlings used in the above experiment appeared to be quite healthy, and there is no reason for supposing that they were not producing root excretion. The failure of the larvae in the mangold cysts to be stimulated by it appears to have been due to the condition of the cysts. It will be noticed that considerably more than half of them were apparently quite dead. The Brassica cysts were in better condition, and one may conclude that they differ from the sugar beet cysts in that the larvae are stimulated by root excretions of certain Brassicas, but not by those of sugar beet.

Another biological difference between the two populations was noticed when attempts were being made to obtain males. The contents of a plant pot in which cabbage seedlings were growing in soil infected with the Brassica eelworm were placed in a Baermann funnel in the hope of extracting males, as mature females had been observed on the cabbage roots. A similar ball of soil containing swede seedlings growing with mangold eelworm cysts, and having mature females on the roots, was placed in another funnel with the same object. When liquid was drawn off from the tubes of the funnels it was noticed that in the case of the mangold eelworm there were numbers of larvae present, whereas larvae were very infrequent in the water from the funnel containing the plants infected with Brassica eelworm. The two balls of soil and plants were left in the funnels for 3 or 4 weeks and whenever water was drawn off from them the same thing was noticed. This seems to show either that hatching may occur from mangold eelworm cysts soon after the cysts are mature, while this does not occur in the Brassica eelworm, or that hatching from the cysts originally in the soil continues for a longer period in the mangold than in the Brassica eelworm.

When examining newly formed cysts it was observed that almost invariably there was present at the posterior end a lump of jelly-like

substance containing from one or two up to 200 eggs at various stages of development, and in some instances also one or two males, which were usually dead (Figure 1). In one or two instances, in some material which had been preserved in formalin, a male was found with the anterior third of the body protruded from the jelly mass and the remainder of the worm coiled up amongst the eggs. It appeared as if the male was escaping from the jelly. The eggs had begun to segment. The presence of such a mass of jelly containing eggs and males is usual in cysts found on the roots of maram grass (Triffitt, 1929b), but is not recorded for the sugar beet eelworm. The occurrence

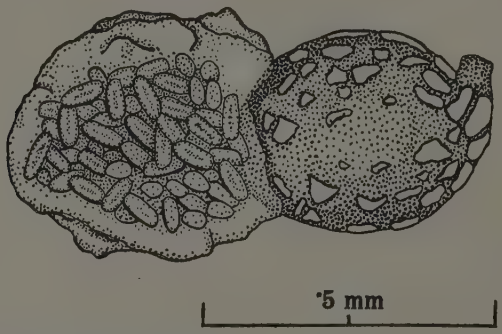

Fig. 1.—Brassica eelworm cyst showing patches of the "sub-crystalline layer" and jelly containing eggs.

of the jelly is not unusual in *Heterodera* (though it is usually less conspicuous), but the occurrence of males in it is curious. In one or two cases the writer has observed a dead male in the jelly attached to a cyst of *H. göttingiana*.

Morphology of the Brassica Eelworm.

Males.—A few males of the Brassica eelworm were examined, but they appeared to be morphologically very similar to those from sugar beet. The stylet measured about 25μ in length, which is a little shorter than in most *Heterodera*. Measurements of the length of males, killed carefully by heat and drawn under the camera lucida, were as follows :—

Brassica eelworm from	broccoli,	6	measurements	... ...	1·200 ± ·0193 mm.
,, ,, ,,	swede	6	,,	... ...	1·177 ± ·0333 mm.
,, ,, ,,	cabbage	2	,,	... ...	1·21 and 1·13 mm.
Mangold ,, ,,	swede	6	,,	... ...	1·443 ± ·0271 mm.

It is seen that the males of the mangold eelworm are significantly longer than those of the Brassica eelworm. Triffitt (1929a) gives mean

lengths of males from sugar beet as 1·46 mm., and from mangolds as 1·42 mm.; she also gives mean lengths of males of the mangold eelworm developed on 14 different hosts. These vary from 1·08 mm. on radish and 1·16 mm. on dock up to 1·64 mm. on sugar beet. It thus appears that there may be considerable variation, and too much importance must not be attached to differences in the lengths of males, especially when small numbers are measured.

Cysts.—The cysts of the Brassica eelworm give the impression of being smaller and much more nearly spherical than those from sugar beet. The vulva is less obvious, but the cysts are definitely lemon-shaped, and appear to have similar irregular " punctations " on the wall. Cysts of the two populations retain their characteristic form and difference in size, even when developed on the same host species, so that anyone who is familiar with them should be able to distinguish a batch of Brassica cysts from one of sugar beet cysts, although individual cysts may, of course, vary from the typical form. The difference in size and shape is illustrated in Figure 2, and the following measurements give the actual size of the cysts :—

Mean length of	122	mangold	eelworm	cysts	from mangolds ...	854·6 ± 8·4μ
,, ,,	100	Brassica	,,	,,	,, cabbage	559·8 ± 14·8μ
,, breadth of	122	mangold	,,	,,	,, mangolds	574·2 ± 6·9μ
,, ,,	100	Brassica	,,	,,	,, cabbage	432·5 ± 5·3μ
,, length ,,	18	mangold	,,	,,	,, swede and cabbage	858·3 ± 28·0μ
,, ,,	14	Brassica	,,	,,	,, swede and broccoli	550·0 ± 22·2μ
,, breadth of	18	mangold	,,	,,	,, swede and cabbage	520·4 ± 17·9μ
,, ,,	14	Brassica	,,	,,	,, swede and broccoli	390·5 ± 15·5μ

According to Triffitt (1929a), mangold cysts averaged 840μ long by 510μ broad, and sugar beet cysts were 730μ by 430μ. The Brassica cysts are approximately the same colour as the sugar beet cysts, and also have a similar " sub-crystalline layer " forming patches of crust on the new cysts (shown in Figure 1).

Larvae.—Measurements of the larvae of the Brassica and mangold eelworms were made using the technique developed by Fenwick and Franklin (1943). The following populations were measured :—(A) mangold eelworm from (*a*) mangolds, 10 larvae from each of 10 cysts from 10 plants (1,000 larvae), (*b*) cabbages, 10 larvae from each of 25 cysts from several plants at random, and (*c*) radishes, 10 larvae from each of 11 cysts from an unknown number of plants. (B) Brassica eelworm larvae were measured from (*a*) cabbages, 10 larvae from each of 10 cysts from 12 plants (1,200 larvae), and (*b*) radishes, 10 larvae

from each of various numbers of cysts (65 all told) from 13 plants. The data were analysed and the analysis of variance is given below.

ANALYSIS OF VARIANCE.

Source	Sums of squares	D. of F.	Variances	Variance ratios
Error (Larvae within cysts)	7,415·600	2,889	2·565 V_1	
Cysts within hosts ...	9,884·217	316	31·15 V_2	$V_2/V_1=12·28$ Sig.
Hosts within eelworm populations	1,105·366	3	368.455 V_3	$V_3/V_4=11·86$ Sig.
Between eelworm populations	25,414·177	1	25,414·177 V_4	$V_4/V_3=69·10$ Sig.
TOTAL	43,819·360	3,209		

It will be seen that the variance between the two populations (V_4) is greater than any of the other variances, indicating a real difference in length between them. The calculation of parameters of the larval lengths will not be undertaken at present, as it is hoped that at a later date more data will be available, and that comparisons may then be made with larvae of other species of *Heterodera*. However, estimates of the means and their standard errors, based on the data so far collected, show that larvae of the mangold eelworm are always longer than those of the Brassica eelworm, even when the two are developed on the same species of host plant, as may be seen from the following data :—

Host	Mangold eelworm larvae		Brassica eelworm larvae	
	Mean length	No. of larvae	Mean length	No. of larvae
Radish	462·5±2·028μ	110	424·1±0·232μ	650
Cabbage	458·9±1·445μ	250	414·6±0·537μ	1,200
Mangold	474·5±0·815μ	1,000	—	—
General	470·6±0·602μ	1,360	417·9±0·519μ	1,850

The standard error of the difference between the two general means given above is 0·7815, giving a value of 91 for " t," a highly significant value. We may thus conclude that there is a real difference in length between the larvae of the mangold and Brassica eelworms.

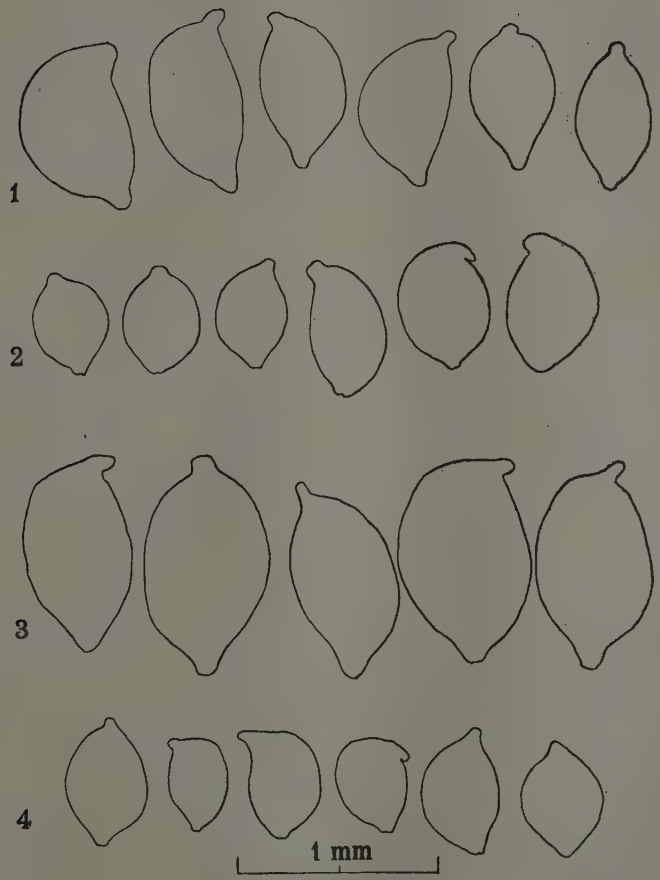

Fig. 2.—1. Cysts from swedes infected with mangold eelworm. 2. Cysts from swedes infected with Brassica eelworm. 3. Cysts from cabbages infected with mangold eelworm. 4. Cysts from cabbages infected with Brassica eelworm

Since the host range and the size of the males, cysts and larvae all indicate that the *Heterodera* found parasitizing Brassicas is different from that known as the sugar beet eelworm, and since the morphology of the males shows none of the diagnostic features characteristic of males of *H. major, H. rostochiensis* or *H. göttingiana,* this Brassica eelworm must be assumed to be distinct from those found on sugar

beet, cereals, potatoes and peas. The differences in the host range between it and the sugar beet eelworm are rather confusing, as both infect Brassicas, but the Brassica eelworm has so far failed, in experiments, to reproduce on Chenopodiaceous plants. The differences may be more clearly appreciated if they are summarized as follows :—

	Sugar beet-mangold eelworm	Brassica eelworm
Larvae ...	Length about 470μ	Length about 418μ
Males ...	Length about 1·4 mm. Stylet 26–33μ (Triffitt)	Length about 1·2 mm. Stylet about 25μ
Cysts ...	About 0·86 by 0·52 mm. lemon-shaped, rather elongated. Eggs and males not normally found in jelly-like mass at posterior end.	About 0·56 by 0·43 mm. lemon-shaped, vulva not always very prominent, general shape broadly oval. Mass of jelly attached to vulva on newly-formed cyst, containing eggs and sometimes males (usually dead).
Host range ...	Infects sugar beet, mangold, spinach, dock, Cruciferous plants, possibly clovers, and numerous other hosts.	Infects Cruciferous plants, but *not* sugar beet, spinach, dock or clover (two tests each).

Specific Status of the Brassica Eelworm.

In the above summary important differences are brought out between the Brassica and the sugar beet eelworms. The most striking biological differences are in the two host ranges, but these by themselves would not carry much weight were it not that there are also quite marked morphological differences. These are most pronounced in the larval and cystic stages, but it is possible that the males of the Brassica eelworm may also prove to be a little smaller than those of the beet eelworm. It is considered that the differences, taken together, are of sufficient weight to warrant the raising of the Brassica eelworm to specific status. The name *Heterodera cruciferae* n. sp. is proposed, as all the plants so far found infected by this species belong to the Family Cruciferae. The specific characters by which the new species is distinguished from *H. schachtii* are as follows :—Males similar but somewhat shorter, about 1·2 mm. long; sometimes found in the mass of jelly attached to the posterior end of the cyst, when they are usually dead. Cysts small, about 0·56 mm. long (excluding the neck) and 0·43 mm. at the greatest width. The larvae appear to be stimulated to hatch from the cysts by root excretions of Cruciferous plants, but

not by sugar beet root excretion; they are small, averaging $417{\cdot}9 \pm 0{\cdot}519\mu$. At the posterior end of the newly formed cyst is a mass of jelly containing eggs and occasionally males. The only known hosts are Cruciferous plants, while in several tests sugar beet, spinach beet, mangolds, red beet and dock (all of them hosts of the sugar beet eelworm) were not infected.

The new species, *H. cruciferae,* is of considerable practical importance: it is a parasite of a large group of widely grown food plants which, although they seem able to support a certain number of the parasites without showing much damage, would undoubtedly suffer if heavily attacked in the seedling stage. If a seed bed should become infected, the eelworm would be very widely and effectively distributed with the seedling Brassicas. From another point of view this species is of practical significance since it is liable to cause confusion in the diagnosis of sugar beet eelworm. The presence of eelworm cysts on the roots of Brassicas does not mean that there is a population present which will attack sugar beet: only an infection on sugar beet can be regarded as certain evidence of the presence of the sugar beet eelworm.

On a Strain of *Heterodera* Attacking Docks and Clovers.

As but little is known of the host range of the *Heterodera* strain occurring naturally on clovers and docks, certain infection tests were made using in all cases cysts removed from the roots of dock (usually *Rumex crispus*) and red and white clovers (*Trifolium pratense* and *T. repens*), mixed with silver sand or sterilized soil. Some experiments were also carried out with cysts of *H. göttingiana* removed from the roots of garden peas. The results are summarised on page 83.

A point which should always be remembered is clearly brought out in these results, namely, that a single negative result may be disproved in further experiments. The results indicate that cysts from clover and dock are probably of the same strain, since both groups of cysts will infect both hosts, and they both attack mangolds, sugar beet and garden peas only with difficulty (most of the " cysts " found on these plants were nothing more than the empty skin of the dead female). Larvae from clover cysts may also attack mustard, but no infection was found on cabbage. This dock-clover strain seems to be potentially capable of attacking a wide range of hosts, and, given the opportunity, might well develop a specialisation for any of them. It may possibly have given rise to the sugar beet race, as the male

spicules resemble those of the latter, and, it will be remembered, cysts from mangolds infected *Rumex crispus*. If the sugar beet race developed from a naturally occurring *Heterodera* infecting clover and docks, it has now become capable of attacking not only Chenopodiaceous plants but also Crucifers. It seems possible that the soil infected with sugar beet eelworm, used in the experiments recorded earlier in the paper, may have contained, in addition to the sugar beet eelworm, a naturally occurring dock-clover strain; this would account for the slight infection on peas and for the infection on clovers. On the other hand these infections may have been caused by the sugar beet eelworm, which is probably not very highly specialised, as suggested by the fact, mentioned above, that cysts from mangolds were found to infect dock. The whole subject is rather complicated, and repeated, careful experiments will be necessary in order to clear it up.

According to the results recorded above *H. göttingiana* seems to have a very restricted host range, which does not, apparently, overlap that of the clover-dock eelworm. The latter is probably best regarded as a biological strain of *H. schachtii,* since the main differences so far observed between it and the sugar beet race are in the host range, and even these differences are not very rigid. In comparing the two strains it is of interest to note that the cysts on clover and dock are of a bright yellow colour before they turn brown, but this stage, if it occurs, is not at all conspicuous in cysts on mangold or sugar beet roots. When investigating the oat eelworm (*H. major*), Goffart (1932) came across the clover strain. He recognised that it differed from *H. schachtii* and considered it to be a variety, which he named *H. schachtii* var. *trifolii*. The strain was described in more detail in 1939 (Franklin). Further investigations are required to discover to what extent it can adapt itself to other hosts.

Acknowledgments are made to D. W. Fenwick, M.Sc., for assistance in the analysis of the data on measurements.

Summary.

1. *Heterodera cruciferae* n. sp., parasitic upon Brassicas and other Cruciferous plants, is described and compared with *H. schachtii,* the sugar beet eelworm.

2. The new species differs from the sugar beet eelworm in its host range, in the reaction of the larvae to root excretions of sugar beet and in the time of hatching of the larvae. The cysts are

Source of cysts	Plant tested	Results
Trifolium repens and *T. pratense*	Mangolds	1. No infection (11/7/41–31/3/42). 2. Two seedlings, stained after five weeks showed one larva (July). Nothing found in other plants after 16 weeks (1/6/43–22/9/43).
	Sugar beet	1. Not infected (7/11/39–16/7/41). 2. One plant out of three had a single young female (15/3/44–20/7/44).
	Rumex crispus	1. No infection (21/6/43–22/9/43). 2. Cysts after 8 months (in March 1944). 3. Cysts after 9 months (in June 1943).
	R. nemorosa	1. Two cysts (16/7/41–31/3/42).
	Cabbage	1. No infection after 9 months (March 1944). 2. No infection after 11 months. (May 1944).
	Garden pea	1. Three larvae after 11 weeks. (July–September) 2. One cyst after 12 weeks. (June–September). 3. Few larvae and young females after 14 weeks. (15/3/44–20/6/44).
	Mustard	1. On four plants one small transparent cyst after four months. (15/3/44–20/7/44).
Rumex crispus ...	Mangolds	1. A few larvae found when roots were stained after three months. (15/7/41–2/10/41). 2. A few empty, transparent cysts on roots after four months. (1/6/43–22/9/43).
	Sugar beet	1. Two small transparent cysts after four months. (15/3/44–20/7/44).
	Trifolium repens	1. A single cyst in eight months. (15/7/41–31/3/42).
	T. pratense	1. Fairly heavy infection (same date).
	Garden pea	1. Not infected in 14 weeks. (15/3/44–20/6/44). 2. A few larvae in 11 weeks. (15/7/41–30/9/41). 3. Young cysts after seven weeks. (18/5/43–5/7/43). 4. Mature cysts after nine weeks. (18/5/43–21/7/43).
Garden pea ...	Broad beans. Infected. *Rumex* sp. (11 months) *Trifolium pratense*. (June–March). Cabbage. (12 months). Sugar beet. (11 months). Dwarf beans. (May–July). Kale, Savoy, Broccoli. (July–April).	No infections found.

somewhat shorter and rounder than in the sugar beet eelworm and differ in having attached to the vulva a mass of jelly-like substance containing eggs and sometimes one or two males. The larvae are shorter than in *H. schachtii*. The males are similar but tend to be smaller and with shorter stylets.

3. The results of infection experiments with cysts from the roots of dock and of clovers indicate that these cysts are of the same race, and are probably a biological strain of the sugar beet eelworm *H. schachtii*.

Addendum.

Petherbridge & Jones (*Ann. Appl. Biol.* xxxi (4) 320-332, Nov., 1944, issued Jan., 1945) refer to two instances of eelworms of the sugar beet type infecting Brassicas but not sugar beet and red beet. They suggest that these infections may be due to a distinct species of *Heterodera*, and it seems highly probable that this is identical with the new one, *H. cruciferae*, described above.

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On some characters of the genus *Trichuris* and a description of *T. parvispicularis n.sp.* from a Cane rat.

By Phyllis A. Clapham, D.Sc.
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The identification of the species of the genus *Trichuris* has been much simplified since the appearance of a paper by Chandler in 1930 in which he pointed out that the prepuce-like expansion surrounding the extruded spicule has very little diagnostic value because of its extreme variability. The length and appearance of the spicule is useful only when used in conjunction with other characters for this is a variable structure. However, the six species with which he was concerned could readily be distinguished by certain internal structures which seem to exhibit great constancy and reliability. These structures are the length of the cloacal tube and its diverticulum for the head of the spicule, the length and shape of the ejaculatory duct and the nature of the folding of the vas deferens and of the testis. Using these characters and other minor ones, he was able to give useful descriptions of the males of *Trichuris trichiura, T. vulpis, T. ovis, T. minuta* and *T. leporis* and to describe a new species *T. tenuis*.

The female is less characteristic than the male but even so there are certain differences in the type of vulva which may be useful in the diagnosis of species when only females are present. Certain characters of the head and oesophageal region, common to both sexes, which do not seem to have been noted previously, will also be described.

All the species examined show a pair of cells apparently glandular, connected with the intestine and lying anterior to it alongside the oesophagus. An actual duct opening into the intestine was not observed in any specimen.

A very large quantity of *T. ovis* was available. The female tended to be somewhat smaller than the male. The head in both sexes is armed with a pair of lateral expansions of cuticle: these seem to be always present in the female in a well developed state but in the male they are sometimes more or less reduced in size—sometimes so much so that considerable search is necessary before their presence can be established. But they are always present. No papillae were seen in any worm of this species.

The bacillary band arises close behind the nerve ring as a narrow structure and gradually widens until at the junction of the oesophageal region with the body proper, it occupies about $\frac{1}{3}$ of the total body diameter, after which it disappears. In the region of the anterior oesophageal glandular cells, the band is provided laterally and sublaterally with prominent cuticular outgrowths or plaques, usually cup shaped or hemispherical and measuring from 25μ to 50μ diameter. There are usually about 25 to 30 on each side, irregularly spaced and not closely packed together.

The male genital system has been fully described by Chandler. The female is characterised by the presence of a very prominent vulva, cuticular in structure and ornamented with small bosses. These vary much in size and shape—they may be so flat as to be almost scales or so elongated as to be little spines, but this ornamentation is always present. It covers the whole of the external surface, sometimes thickly, sometimes rather sparsely, and is continued for a short distance along the internal channel of the vulva. The whole structure is very prominent, sometimes attaining a length of 200μ and overhanging the side of the worm in a striking manner. The anterior portion tends to be rather more prominent than the posterior.

Trichuris trichiura was available in a fair quantity from a number of primate hosts from various parts of the world. The variety *suis* was not available in this sample of material. Females can be distinguished from those of *T. ovis* by a number of characters. The head never possesses lateral wings in either sex : it is a bluntly rounded structure which widens quite suddenly at the level of the nerve ring.

Figs. 1–4. *Trichuris ovis.*

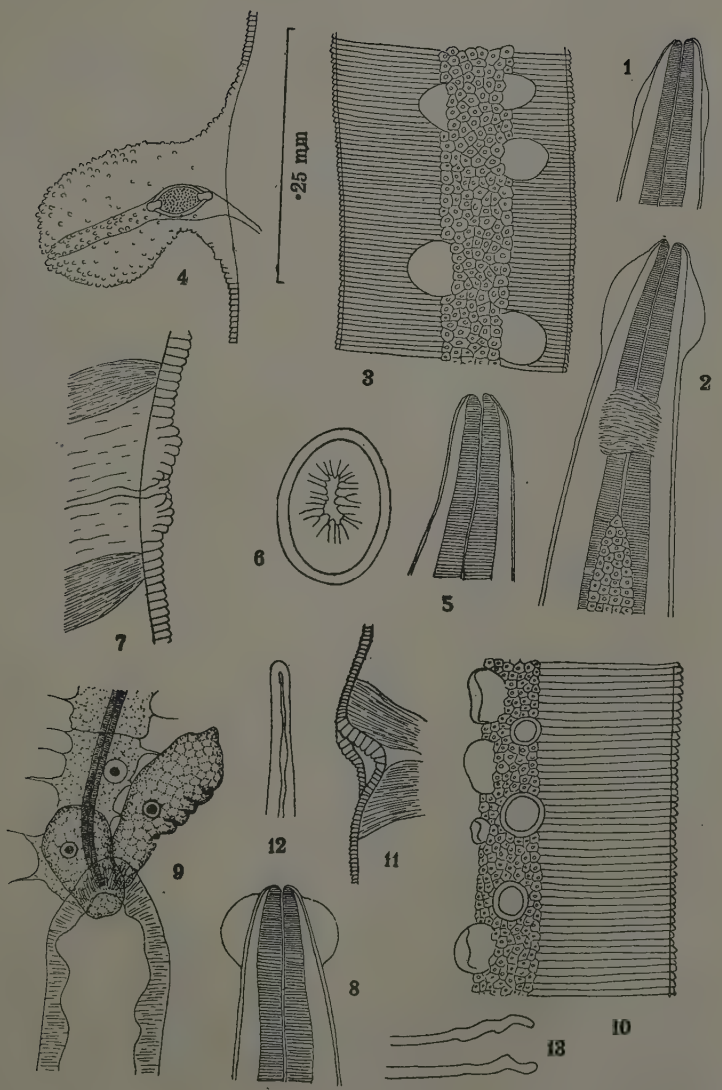
Fig. 1.—Head of male.
Fig. 2.—Head of female, showing wings, nerve ring and the beginning of the bacillary band.
Fig. 3.—Bacillary band in ventral view.
Fig. 4.—Vulva.

Figs. 5–7. *Trichuris trichiura.*

Fig. 5.—Head of female—this is indistinguishable from that of the male.
Fig. 6.—Vulva in surface view.
Fig. 7.—Vulva in lateral view.

Figs. 8–13. *Trichuris parvispicularis.*

Fig. 8.—Head of female—indistinguishable from that of the male.
Fig. 9.—Anterior region of the intestine, showing the pair of glandular cells and the opening of the true oesophagus.
Fig. 10.—Bacillary band in lateral view.
Fig. 11.—Vulva in lateral view.
Fig. 12.—Spicule—distal tip.
Fig. 13.—Spicule—proximal tip.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In some specimens there appeared to be a pair of lateral papillae but these were not seen in all and so may be an artifact.

The vulva is distinct. Here there is no protruding outgrowth overhanging the edge of the worm. It is placed on a slight prominence, the anterior side of which tends to be a little more marked than the posterior. The aperture is transverse when not distorted by an egg. The cuticle round the aperture is coarsely striated in a radial direction, the whole being surrounded by a ring of clear cuticle, so that in surface view the general appearance resembles that of an iris diaphragm. Sometimes the radial striations may be complicated by subdivision giving finally a rosette appearance but the ornamentation never takes the form of bosses.

Cuticular plaques are present sub-laterally in the bacillary band in both sexes at the level of the anterior oesophageal glandular cells. There are 30 to 35 on each side and they develop in close apposition to each other forming an almost continuous line on each side. Though varying in size and shape to a certain extent, they measure on an average about 20μ to 30μ in diameter and are usually cup-shaped in design.

Trichuris parvispicularis n. sp. was collected from the caecum of a cane rat, probably *Thryonomys swinderianus* in Southern Rhodesia in 1930. The material consisted of 4 males and 6 females of which only 3 were intact. The bodies of the males were all tightly coiled in overlapping spirals with the result that accurate body measurements were not easy to obtain. The total body length seems, however, to vary between 58 mm. to 72 mm., with a maximum diameter of about 700μ, the oesophageal region accounting for about $\frac{2}{3}$ of the total length. The females were fairly straight and the intact ones measured respectively 56, 63 and 68 mm. long. The oesophageal portions of these worms measured 37, 45 and 43 mm. long, the proportion being again about $\frac{2}{3}$ of the total.

The head in both sexes is bluntly rounded and measured about 60μ at the level of the lateral alae which measure about 60μ long by 25μ across. They are equally well developed in both sexes. The bacillary band is provided with sub-lateral plaques in vast quantity. They number up to 60 on each side and are irregularly scattered, beginning in the very early part of the band before the level of the oesophageal cells and extending for some considerable distance along the thin part of the worm. They vary much in size, the early ones tending to be

the largest and these may measure up to 50μ in diameter. They are all cup-shaped in outline.

As the males were so tightly coiled, it was necessary to dissect out the genitalia of some, for clearing, even in phenol, was not effective. The spicules in three specimens were fairly short and measured respectively 2·26 mm., 2·175 mm. and 2·28 mm. long. The free tip is very blunt in all specimens and in this region the spicule measures about 25μ across. It broadens gradually until it measures nearly 50μ across and terminates in a funnel shape. The spicular sheath is very variable in outline and when extended may be a simple tube or may expand into a terminal or sub-terminal bulb distally. It seems to be covered all over with small spines. The distance from the end of the body to the union of the ejaculatory duct with the spicular caecum varies from 0·93 mm. to 1·18 mm., while the distance from the end of the body to the union of the intestine with the vesicular seminalis varies from 1·17 mm. to 1·87 mm. The testis is loosely coiled in wide sweeps which stretch across the whole diameter of the body : later they become more compact. The vas deferens, too, is loosely coiled. The ejaculatory duct is not highly muscular but is a fairly coarse wide tube. The intestine is narrow, being only about $\frac{1}{8}$ of the body width.

The female is slightly bent but does not show any of the intricate spiralling of the male. The vulva is in the usual position just behind the beginning of the intestine. The opening lies flush with the general contour of the body surface and in full face view is usually three sided with the long base transverse and the point facing posteriad. It is surrounded with thickened cuticle decorated with coarse striations. The anterior side forms an overhanging lip and here the cuticle is much thickened and striated. The eggs are not distinctive measuring from 62μ to 86μ long by 27μ to 31μ broad, including the apical plugs. These are rather prominent, resembling those of *T. ovis*.

The specific name *parvispicularis* is suggested because the spicules are not particularly long.

Host—Cane rat, probably *Thryonomys swinderianus*.

Position—Caecum.

Geographical distribution—Southern Rhodesia.

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Some Helminths from West Africa.

By Phyllis A. Clapham, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans.*)

The material described consists of two small collections. One composed mainly of helminths from birds, was collected by Major T. A. Cockburn, M.D., D.P.H., on the Gold Coast in 1943. The other was collected from mammals by Dr. K. Morris, of the medical department, Kumasi, in 1939. To both of these collectors and to Dr. R. T. Leiper, C.M.G., F.R.S., who kindly put the material at my disposal, I am much indebted.

The bird material contained 8 different genera, of which one is a trematode, three are cestodes and the others are nematodes. The Acanthocephala are not represented.

A Squacco Heron, *Ardeola ralloides*, yielded a number of specimens of the trematode *Clinostomum complanatum* (Rud., 1814), Braun, 1899. They were taken from the oesophagus where they were actively moving about at the post-mortem examination. It is a small worm between 3 and 4 mm. long, strongly muscular with comparatively large suckers. The vitellaria are abundant and so deeply staining as somewhat to obscure the other organs. It has previously been recorded as a parasite of *Ardea cinerea* and *Nycticorax griseus* in Europe, but apparently both this host and this geographical distribution are new records. The vector is a fish; Ciurea in 1911 having implicated *Perca fluviatilis*.

Two specimens of *Streptopelia senegalensis* harboured an infestation of *Raillietina crassula* (Rud., 1819). This is a common parasite of a number of Columbiform birds and has been recorded from Europe, Africa and S. America. It is usually assigned to the sub-genus *Fuhrmannetta* on account of the genital openings being irregularly alternate and the egg capsules containing several eggs each. It is a large species attaining a length of up to 40 cm. Though the present material is shorter it is fragmented so that the actual length is not possible to measure. But the worms are mature and contain ripe eggs. There are about 70 rostellar hooks 20μ long and a large number of small hooklets on the suckers.

One of these birds also contained in its small gut two specimens of *Hymenolepis serrata* Fuhrmann, 1906, another species which is known to parasitize Columbiform birds. Neither worm is gravid but they can be recognised by the following characters: the small scolex

has oval suckers and the rostellum is not armed. The three testes lie in a single line and there is a very large cirrus sac stretching more than half way across the segment. It contains a prominent vesicular seminalis and a long cirrus. There are small spines surrounding the genital aperture. The ovary is small and broad and lies behind a wide hour glass shaped receptaculum seminalis. *H. serrata* has not been recorded either from this host or from West Africa before.

One of the tubes in this collection contains a species of *Choanotaenia,* the host of which is a little uncertain but which is probably a West African Harrier Hawk, *Gymnogenys typicus pectoralis.* The worm itself is broken up into a number of small fragments and the scolex is missing so that it is not possible to assign it to a definite species. The segments, however, show considerable resemblance to those of *C. polyorchis* which is a parasite of a number of species of *Milvus,* but the present material and available information make it inadvisable to be too definite about the species. The mature segments contain 30 to 40 testes, oval in outline and about 60μ across. The cirrus sac is coiled upon itself. A receptaculum seminalis is present lying behind the cirrus sac. The mature segment is longer than it is wide.

Among the tubes containing nematodes is one holding a single specimen of *Lagochilascaris,* from an unknown host but probably another Harrier Hawk. This is a genus of mammalian parasites, no species having been recorded from birds. Carnivores are the usual hosts. As the worm shows signs of considerable decomposition it is possible that it had been taken in with the food and was in process of digestion. Its presence, however, in Africa and in the Gold Coast is interesting for there is only one previous reference to its occurrence in the Old World when *L. major* was recorded by Leiper in 1909 from *Felis leo* on Kilimandjaro. The specimen shows all the characters of the genus but is too decomposed for the recognition of specific characters. There are three lips followed by a deep cephalic groove behind which is the thick chitinous ring from which arise the three interlabia. The vulva is in front of the middle of the body and a few eggs are available. These have the usual thick shell with mosaic decoration found in other *Lagochilascaris* species.

The Anisakinae are represented by a single female specimen of a species of *Porrocaecum,* probably *P. depressum* (Zeder, 1800) Baylis, 1920, taken from the gut of a vulture. It is a young adult, but ova are not yet formed. The species has been recorded from various kinds

of vultures but has not been noted in Africa further north than the Transvaal.

The only Filariid worms are two females of a species of *Diplotriaena*, one of which is damaged. Without males, however, one cannot differentiate the species for the females are not distinctive morphologically. The trident-like structure of chitinous material at the anterior end of the oesophagus is clearly visible and the vulva is close behind the muscular portion of the oesophagus. Both worms are gravid and contain a very large number of embryonated eggs. The genus is largely composed of bird parasites, many species having been recorded from a great variety of birds. The geographical distribution is wide. These specimens were found in the peritoneal cavity of a Buff backed Egret, *Bubulcus ibis*.

This collection also contained specimens of *Porocephalus* from a Gaboon Viper.

The mammalian portion of the collection consists of 6 tubes of which 5 were collected from Roan Antelopes. Three of these contain cestodes which are identified as *Stilesia globipunctata* (Rivolta, 1874) Railliet, 1893, though the scolices are missing. The worms are mature and the uterus is apparent in the gravid segments with its peculiar structure filled with eggs. This species has been recorded from a large number of antelopes and is already known to occur in the Gold Coast. This material was collected in the Gambaga region.

Nematodes are represented by two species (*a*) *Setaria labiatopapillosa* (Alessandrini, 1838) Railliet and Henry, 1911, is a common parasite of deer and various antelopes and has a cosmopolitan distribution. The present material was taken from the peritoneum of Roan antelopes.

(*b*) *Physaloptera præputialis* Linstow, 1889 from the stomach of a lion. It is already known to occur among felines in Africa and other parts of the world.

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Some bird Helminths from Antigua.

By Phyllis A. Clapham, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans.*)

The material on which this article is based was sent to Dr. R. T. Leiper, C.M.G., F.R.S., by Mr. L. R. Hutson, Veterinary Officer, Antigua, to both of whom my thanks are due. Most of the cestodes had already been described by Prof. J. G. Baer and recorded by him in 1940. The rest of the collection consists mainly of nematodes but there are, however, representatives of all three groups of helminths—Trematoda, Cestoda and Acanthocephala as well as the Nematoda. The hosts were all described by the local popular names and I am much indebted to Mr. Kinnear, of the Natural History Museum, London, for identifying them.

The only trematode in the collection is the Strigeid, *Apharyngostrigea cornu* Goeze, 1800, which had been removed from the large gut of a Yellow crowned night heron, *Nycticorax violaceous*. It has already been recorded from a number of birds belonging to the Ciconidae and to the Ardeidae but not from this particular host. It is not uncommon in Europe and the United States but has not been reported in the British West Indies before. There are two specimens both attached to the mucous membrane but they were easily removed and seemed to have caused no pathological changes. They are mature and contain eggs and the vitellaria are strongly developed, obscuring most of the other organs.

A single tube of cestodes had been left unidentified. It contained typical specimens of *Diorchis americana* Skrj., 1914, which had been taken from the large gut of a Red Seal Coot, *Gallinula catoropus*. The species has already been reported from this genus.

Oxyspirura mansoni (Cobbold, 1879), Ransom, 1904, from the eye of a chicken is no new record. It is of cosmopolitan distribution. It was represented in this collection by 2 females and 1 male.

Dispharynx sp. occurred in one of the tubes, a single immature specimen having been taken from the oesophagus of *N. violaceous*. It may have been *D. spiralis* but development was not sufficiently far advanced to be certain of the species. The worm was taken from the same heron as harboured the trematode.

The oesophagus of another *N. violaceous* contained a species of *Echinuria*. A portion of the organ had been sent and from it 26

helminths had been removed but as none are mature, they have not been assigned to any particular species. Most are sheathed larvae but two of them have shed their sheaths and are apparently pre-adults. One is male and the other female.

The male measures 6·34 mm. long by 0·20 mm. wide at the level of the intestine. The cuticle is finely striated transversely and each cordon passes backwards for a distance of 928μ before uniting with its fellow from the other side. They follow a wavy course and seem to consist of a clear central region with lateral teeth projecting backwards on either side, making a total width of 8·5μ. The tail carries 3 pairs of pre-anal and 4 pairs of post-anal papillae: the spicules are not heavily chitinized. One is curved and measures 400μ long, while the other is almost straight and measures only 60μ long. There are no caudal alae. The tail is spirally coiled. Anteriorly there are two lips, each with three papillae, the central one being comparatively prominent. The mouth pore leads into a cylindrical buccal cavity 22μ long, followed by the double oesophagus. The muscular portion measures 0·839 mm. long while the glandular portion is 2·16 mm. long.

The female measures 6·31 mm. long by 0·223 mm. wide at the level the intestine. The cuticle and cordons are like those of the male. The paired lips each have three papillae and the mouth pore leads into the buccal cavity. The two portions of the oesophagus measure 0·65 mm. and 2·186 mm. long respectively. The vulva is posterior, slightly in front of the anus. The tail has a short chitinous point and is turned ventrally. The genital tubes are simple. There is no evidence to show what stage of development these larvae have reached: it may be that a further moult is necessary.

The rest of the larvae are sheathed with no suggestion of sex differentiation. They vary in length from 5·65 mm. to 6·0 mm., including the sheath, with a width of from 0·162 mm to 0·22 mm. There are two small lips each with an apical papilla, no others being visible. The buccal capsule measures about 20μ long and the two portions of the oesophagus are from 0·5 mm. to 0·72 mm. long and from 2·10 mm. to 2·30 mm. long respectively. The cordons are developed and show the typical structure. They are apparent not only on the larvae but also on the surrounding sheaths. Thus the cephalic decoration develops before the final moult, at least on the 4th stage larva.

Most of these larvae were lying free on the surface of and among the folds of the oesophagus. A few had, however, buried their heads in the tissue which had become slightly hypertrophied as a result. A small degree of round celled infiltration was present.

There were several other spirurid worms. Between the tunics of the gizzard of a Greater Yellow Legs, *Totanus melanoleucus*, a single mature female of a new species of *Yseria*, for which the name *Y. quadripartita* n. sp. is suggested, was obtained. It is a stout worm, black in colour and very dense so that its anatomy is not easily seen even after drastic clearing in phenol. It measures 10·09 mm. long by 160μ wide in the intestinal region. The body tapers gently at the anterior end and sharply behind the anus. The cuticle is striated. The oral opening is elongated dorso-ventrally and is provided with two small lips each with a pair of sub-median papillae and a cephalic tooth. The head is decorated with two pairs of cuticular outgrowths, directed backwards and each divides into four finger-like processes. The cylindrical buccal cavity is about 80μ long. The two regions of the oesophagus measure 410μ and 1·5 mm. long respectively. Posteriorly the tail turns dorsally and is blunt. The vulva opens slightly behind the middle of the body, being 5·4 mm. from the anterior end, and is connected with a muscular vagina, directed backwards. This in turn is formed by the union of paired uteri which pass, anteriorly to the level of the glandular oesophagus and posteriorly to the level of the rectum, where they turn back. The ovaries are solid and lie in the middle region of the body. The uterus is full of eggs with a smooth, clear shell and measure 33μ by 20μ. The embryo appears to segment within the uterus and to become embryonated before passing out.

The worm is much smaller than either *Y. californica* or *Y. coronata*. Further, *Y. californica* has three teeth on the cervical papillae and the vulva is behind the middle of the body; in *Y. coronata* there is a single tooth on the cervical papillae and the vulva is almost median. Here the vulva is posterior to the middle of the body and the papillae carry a single tooth. Furthermore the cephalic flaps are subdivided into 4 processes.

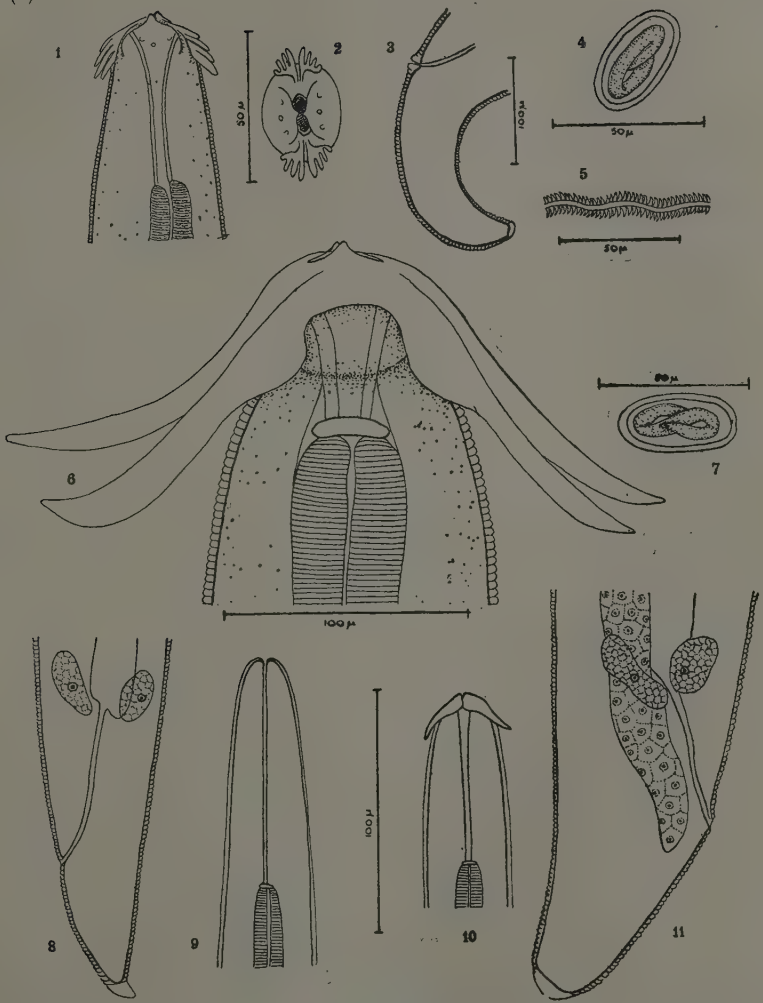
The gizzard of a Willet, *Catoptrophorus semipalmatus* yielded some female worms of the genus *Schistorophus* and a large number of spirurid larvae, which are not, however, sufficiently distinctive to allow them to be assigned to any particular species or genus. The same adult was also recovered from the gizzard of *Himantopus mexicanus*,

the black necked Stilt. Spirurid larvae, obviously young forms of a species of *Schistorophus* were obtained from the gizzard of a Rufous necked Plover, which has been identified by Baer as *Oxyechus vociferus*. These forms will be considered together. All the worms were lying between the tunics of the gizzard and were liberated simply by separating them. The adults measure from 12·8 mm. to 13·6 mm. long and they are gravid. The body width varies from 270μ to 310μ at the level of the beginning of the intestine : it remains very constant there being only a slight tapering anteriorly and a rather sudden one behind the anus. The cuticle is striated transversely, only the final tip of the tail being clear. The head is decorated with 4 bluntly pointed cuticular festoons, uniting anteriorly with the cuticle, joined at their origin and carried over the head. The mouth is small, elongated dorso-ventrally, and armed with a pair of teeth. It leads into a cylindrical buccal cavity, strongly chitinized and resting on a well marked ring of chitin. It narrows somewhat posteriorly, thus becoming funnel shaped. It is 50-50μ long by 33μ wide at the opening and 20μ wide at the junction with the oesophagus. This is in two parts : the anterior muscular part is about 840μ long while the glandular part is slightly shorter. The nerve ring surrounds the muscular part about a third of the way down. The intestine is not interesting and it ends in a rectum associated with a pair of rectal glands. The anus is sub-terminal opening about 80μ from the end of the body which turns very sharply dorsally.

The vulva is not prominent and lies shortly behind the middle of the body. It leads into a backwardly directed vagina and the genital tubes are paired and opposed. The ovaries lie in the regions of the glandular oesophagus and of the rectum and oviducts appear to be absent for the ovaries open abruptly into the uteri. The oldest eggs are embryonated. They measure 48μ long by 27μ broad and are provided with a thick clear shell without any marking or decoration.

In the absence of male worms, it is difficult to place these females into a definite species. Charadriiform birds act as hosts to a number of *Schistorophus* species but the position of the vulva excludes the possibility of *S. longicornis,* while the female of *S. aulieatina* is twice as long as the present species. There is some resemblance to *S. laciniatus,* reported from *Rallus cayennensis* in Brazil, and this material will therefore be considered as belonging to this species.

The larvae that are associated with these adults are in two stages of development. The younger ones measure from 3·93 mm. to 4·82 mm.



Figs. 1 to 4.—*Yseria quadripartita*.—Fig. 1. Head—lateral view. Fig. 2. Head—end-on view, somewhat diagrammatic. Fig. 3. Tail of female. Fig. 4. Egg from uterus.

Fig. 5.—*Echinuria sp*.—Cordon from larva.

Figs. 6 and 7.—*Schistorophus laciniatus*.—Fig. 6. Head—lateral view. Fig. 7. Egg from uterus.

Figs. 8 to 11.—Schistorophid larvae.—Fig. 8. Tail of youngest larva. Fig. 9. Head of youngest larva. Fig. 10. Head of older larva. Fig. 11. Tail of older larva.

long by about 60μ broad. The body tapers anteriorly, less so posteriorly. The cuticle is finely striated: the divisions being about 6μ apart. In most of the larvae the cuticle is of an even thickness but in a few there seems to be a slight hypertrophy round the mouth pore.

A dozen larvae were measured: the buccal cavity measures from 73μ to 104μ long. The muscular oesophagus varies in length from 325μ to 390μ while the length of the glandular oesophagus appears to be from 670μ to 690μ. The anus opens about 60μ from the tip of the body. No genital glands are visible.

The older larvae are larger reaching a length of from 6·3 mm. to 6·6 mm. The buccal cavity is shorter than in the previous forms, being only 59μ long but it is broader and sturdier. The muscular oesophagus measures about 0·5 mm. long and the glandular oesophagus is 1·58 mm. long. The cuticular striations are wider than in the young larvae, about 8-10μ apart and they are more marked. The genitalia are not yet developed as such but a group of glandular cells rather behind the middle of the body can be seen, which are certainly the beginnings of the genital system.

The spirurid larvae taken from the gizzard of *O. vociferus* are still further developed and can be recognised as belonging to the genus *Schistorophus*. They are very constant in size, measuring about 9 mm. long by 120μ wide. Anteriorly the body tapers in the oesophageal region but posteriorily it only narrows appreciably behind the anus. The cuticle is lightly striated.

The cephalic decoration is beginning to develop and appears as a pair of festoons which have met in the middle line but the line of union is still visible: there is no unbroken expanse of chitin as in the adult. Lips were not seen. The buccal cavity is cylindrical only very slightly wider at the pore than lower down. It measures 62μ long. The muscular oesophagus is 1·09 mm. long and the glandular oesophagus measures 1·73 mm. long. The intestine is uneventful and is lined with columnar epithelium with large nuclei. The genitalia is developing and appears as a pair of opposed tubes which extend the length of the body from the oesophagus to the rectum. The ovaries are formed of large nucleated cells: the uteri are practically straight though in a few worms a few convolutions can be seen. The vagina is directed backwards and is composed of large granular cells. The whole structure is solid and the vulva is not yet perforate. It will,

however, open eventually behind the equator, dividing the body into the proportions 10 :7.

The Acanthocephala are represented by two species. The large gut of *Nycticorax violaceous* yielded several specimens of *Corynosoma semerme* (Forsell, 1904) Lühe, 1911. This is a very small species, already reported from a number of fish-eating birds in Europe. Its presence in this host and in the British West Indies has not been noted before.

There are also two tubes containing Acanthocephalan worms from the large gut of the Pigeon Hawk, *Falco columbarius*. These are identified as *Centrorhynchus globocaudatus* (Zeder, 1800). It has been recorded previously from a number of birds of prey in Europe and probably in S.W. Africa but not from this particular host or district before.

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A curious case of nematode parasitism in a West African Hadada, *Geronticus hagedash.*

By PHYLLIS A. CLAPHAM, D.Sc.

(*From the Institute of Agricultural Parasitology, St. Albans.*)

IN the following article is described an interesting parasitic condition which is difficult to interpret. The small intestine of an Hadada, *Geronticus hagedash,* was brought back from the West Coast of Africa by Major T. A. Cockburn, M.D., R.A.M.C., who kindly passed it to me for further examination. The bird is a member of the family Plataleidae, living in wooded districts in West Africa in the neighbourhood of water and feeding on invertebrates, mainly annelids and small crustaceans which it finds at the bottom of ponds and streams in the mud.

The portion of the intestine available for examination is heavily encrusted with small nodules—there are 52 on a piece of gut measuring only 15 cm. long. They are irregularly dispersed, sometimes singly, sometimes in groups of 5 or 6. Strongly outstanding on the external surface of the gut and measuring from 2-2·5 mm. diameter, they lie in the external muscular coat, the inner mucosal lining being intact. When these nodules are opened up, the contents which escape are seen to consist entirely of embryonated eggs and hatched larvae of some nematode, together with a small quantity of caseous matter. Each nodule contains several thousands of such nematodes. The eggs are large, measuring from 64μ to 72μ diameter: they are mostly spherical but some have become elongated, either by compression within the cyst during fixation or by the active movements of the contained larvae. They then become long ovals with very blunt ends. Such ova measure as long as 110μ by 45μ to 50μ wide. The shell is very thin, transparent and apparently pliable. Some of the ova have hatched and the resulting free larvae measure 328μ to 350μ long by about 24μ wide in the region of the intestine. The cuticle is smooth and the tail tapers to a point which is often turned dorsally. There is no sheath. The mouth is a simple pore and leads directly into a simple muscular oesophagus 85μ to 90μ long. The intestinal cells are difficult to see as they are full of oily globules. These larvae have no distinguishing features.

But in each nodule there is also a single larva in a more advanced stage of development: the conditions are precisely the same in every nodule examined. This larva is a female which has apparently undergone her last moult for the vulva is perforate. She measures from 2·0-2·4 mm. long and has a smooth cuticle. The mouth is a simple pore leading into a muscular pharynx which measures 92μ to 115μ long. This is followed by a glandular oesophagus measuring 285μ to 415μ long. The intestine is curiously folded back over the end of the oesophagus causing it to become funnel shaped. It opens subterminally through the rectum. The genitalia are well developed. The vulva opens shortly behind the middle of the body about 0·8-1 mm. from the posterior end. It opens into a muscular vagina which is connected with paired, opposed reflexed genital tubes. The uteri are thin walled, measure from 363μ to 440μ long and are deliminated from the oviducts by thick walled tubes each with a sphincter. Each oviduct is long and muscular, reaching to the levels of the oesophagus and rectum respectively where it bends on itself, passes medially and becomes the

solid ovary. The tips of the ovaries lie in the region of the vulva. But the whole system is immature. The ovaries are obviously not functional and there are no ova in the oviducts. These structures in fact

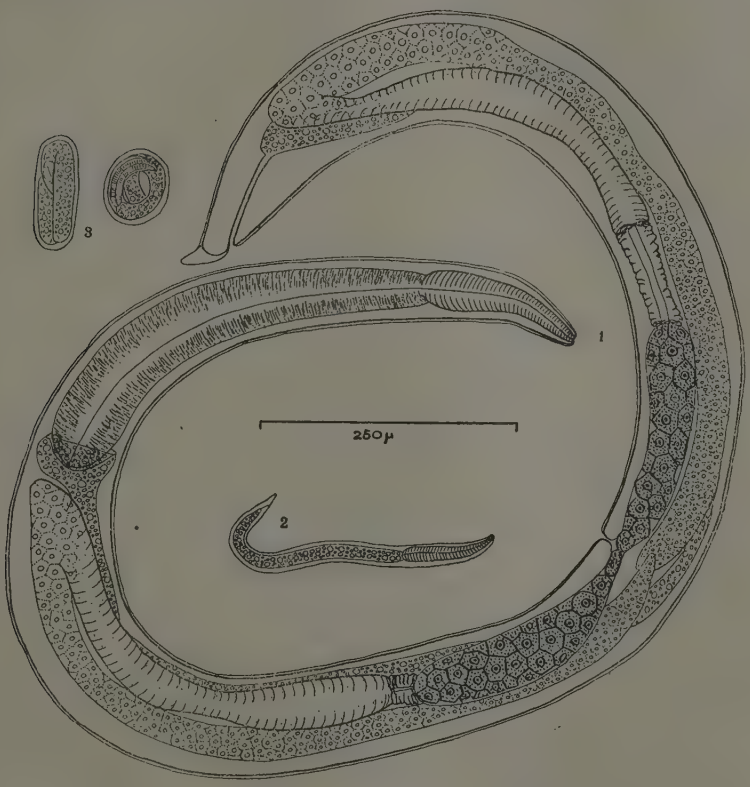

Fig. 1.—Immature female spirurid worm showing uterus full of sperm and non-functioning gonads.

Fig. 2.—Young larvae from nodule.

Fig. 3.—Embryonated eggs from nodule.

appear to be quite empty. But the uterus is full of sperm and seems to be acting as a receptaculum seminalis.

These facts are difficult to interpret. The more developed larva is probably a spirurid as judged by the nature of the gut with its muscular

pharynx and glandular oesophagus. Many spirurid worms are parasites of birds where they often live in some portion of the gut in their adult state. Some occur in the lumen but a number penetrate into the substance of the gut wall and live in close contact with the tissues. In some cases they are in even more distant locations. In every case, however, the ova, usually embryonated, are liberated into the lumen of the gut and pass out to the exterior to undergo a period of development within the body of a suitable vector which is usually a small crustacean. They rarely hatch before they are ingested by the intermediate host, except in the case of certain species of *Habronema*. There is usually a choice of vectors though specificity is the case with some parasites of this group. After ingestion the larvae hatch, moult twice and then remain dormant as infected larvae, often becoming encysted. Transport hosts are fairly common among the spirurids. For instance if the larvae are taken up by an unsuitable host, they frequently survive, pass into the tissues of the gut and encyst again awaiting ultimate access to a suitable definitive host. By then the larvae have reached the infective stage and are encysted singly not in groups of several thousands as occurs in this bird.

No adults were found anywhere, either in the lumen of the gut or in its tissues, and there is no morphological or pathological evidence that any have ever lived in this stretch of gut. The female in each nodule is too small and too immature to have given rise to the numerous eggs and larvae that surround her. The nodule itself is not the swollen and engorged body of a female worm such as occurs in the genus *Tetrameres*, and it is as difficult to see how such vast numbers of eggs and larvae came to be placed in these circumscribed areas as it is to understand how they can hope to be liberated to the exterior and their intermediate host except by the death of the definitive host and its subsequent decomposition in some place where small crustaceans abound. Such a method would be very hazardous and wasteful of larvae.

The presence of the immature female is even more difficult to explain. She may be a precocious female belonging to this batch, but it is not usual for spirurid worms to develop to the stage of the last moult without an intermediate host. Further, one would expect more than one such larva per cyst and not all should be females. There is no trace of a male worm within the nodules, yet she contains spermatozoa. The male may have been missed during the examination or he may

have died and disappeared following insemination or finally the female may be protandrously hermaphrodite. The examination of twelve nodules was extensive and minute, and it is difficult to believe that a male worm, even though small, could have been missed each time. It is, however, equally difficult to see how he could disappear in a short space of time in such a confined area without leaving some evidence of his previous presence. He may of course have been a small form living in the lumen of the gut which had escaped notice by the collector, but in that case we have to account for the entry of the female into these nodules. Finally to consider the case for protandry—it is not unknown among nematodes, particularly among the free living forms, but it is less common among the parasitic genera. If this is a protandrous female, she must first have matured without the intervention of an intermediate host, and secondly one would expect to see signs of activity in the gonads and a trickle of spermatozoa in the oviducts. But the sperms are limited to the uterus, the oviducts are empty, and there is no evidence that the gonads are functional.

It is of course not certain that these eggs and larvae belong to the same adult as the immature female, but it would be rather astonishing if two unrelated worms should happen to choose precisely the same inaccessible site for propagation.

Spirurid worms are known to be versatile in their life history and to be able to withstand abnormal conditions, e.g. they can remain viable in abnormal hosts and develop naturally when they obtain access to a normal one. It may be that in this case now recorded, the hadada is an abnormal host to which unusual surroundings the parasites have reacted in a peculiar manner. But no attempt is made here to formulate a theory that will explain all these curious data or to diagnose specifically the worms that have been found. But the whole condition is so strange that it has seemed worth while to bring it to light as it may prove interesting and perhaps less incomprehensible to other parasitologists.

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An Alien Weed Host of *Heterodera rostochiensis* in England.

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Many crops of carrots grown in gardens and on a field scale, during the War, have been infested with a weed which bears a resemblance to *Solanum nigrum*. It has been identified as *Solanum sarachoides* Sendtner. The plant is a native of Temperate and Sub-temperate South America. The carrot seeds containing this impurity are said to be of Californian origin.

The plant possesses oval leaves, slightly more wavy than those of British specimens of *S. nigrum*. They are smooth and dark green in colour, but the stems are covered with short hairs. The habit is procumbent to prostrate and the weed creeps extensively through the dense foliage of the carrot crop. The inflorescences are borne laterally and display minute white flowers. Dark green berries are produced in great profusion each containing about 25 seeds. There is evidence that the plant is becoming established on light soils, in the market gardening districts of Staffordshire, and it is doubtless localised elsewhere.

It was noted that where the weed was growing adjacent to plots of potatoes affected with blight (*Phytophthora infestans*), that its leaves were also attacked. The lesions resembled those of potato blight and the identity of the fungus was confirmed by Mr. N. C. Preston, of Harper Adams Agricultural College.

During the present summer the weed was grown in six separate pots each filled with soil known to be infected with *H. rostochiensis*. Potatoes were grown in six similar pots for a comparison. The roots of the weeds and of the potatoes became highly infested with cysts. The plants presented a sickly appearance, the leaves took on a yellow tinge and there was some necrosis and twisting of the lamina.

An attempt has been made to discover evidence of infection in the field, but so far no case has been found where the weed occurs on land infected with *H. rostochiensis*. There appears to be no reason why preventive measures should not be taken by proper cleaning of consignments of carrot seeds. The seeds of *S. sarachoides* are similar in size and shape to those of the potato, and owing to their great differences in these respects from those of the carrot, there should be no difficulty in removing them.

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Anguillulina brenani n.sp., a Nematode causing Galls on the Moss, *Pottia bryoides* Mitt.

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Up to the present time, so far as the writer is aware, there has been no previous British record of galls on a moss caused by a nematode although cecidologists have reported galls of nematode origin on 52 species of moss on the continent of Europe (see Goodey, 1940). Except in the case of *Hypnum cupressiforme* L. and *Thuidium delicatulum* (L.) Hedw., in which the causal parasite is *Anguillulina askenasyi* (Bütschli, 1873), the specific identity of the worms affecting the other 50 species has not been determined though it is probable that in all cases they belong to the genus *Anguillulina*.

In spite of Bütschli's original differentiation of *A. askenasyi* from *A. dipsaci*, which in some features it closely resembles, Bos (1888) made it a synonym of the latter and in this position it remained until Steiner (1936) re-established it as a distinct and valid species; basing his description on nematodes found causing terminal shoot galls on the fern-moss, *Thuidium delicatulum* (L.) Hedw., occurring in West Virginia, U.S.A. In view of these circumstances it was with considerable interest that the writer undertook the examination of galls on the moss, *Pottia bryoides* Mitt., sent to him by Mr. J. P. M. Brenan of Oxford, who had discovered nematodes in the galls and requested the writer's help in determining their identity.

Two lots of material were received, the first at the end of March and the second about mid-June, 1945. In both cases it consisted of living specimens of *Pottia bryoides* bearing shoot galls which Mr. Brenan had collected from rough, dry calcareous soil close to Headington Wick Copse, Oxford.

Pottia bryoides is a very small moss, the shoots in many cases being only 2 to 5 mm. long. The swollen, somewhat pyriform galls (fig. 1) occur at the tips of main or of branch shoots in place of a tuft of leaves as in a healthy shoot (fig. 2.) The galls are very small and vary in size from 0·5 mm. to 0·75 mm. long by 0·3 mm. to 0·45 mm. in width at the widest part. Young galls are green but older ones are deep brown in colour. By the careful dissection of galls under the microscope it was found that each consists of a number of leaves which enclose

a small hollow cavity containing the nematodes. The latter usually include one or two adult males and females and, in older galls, some eggs and larvae in various stages of growth. The writer has never found more than 2 adult males and 3 adult females in a single gall. The females are always more or less coiled ventrally, thus resembling the naturally curved habit of the females of *A. askenasyi,* as described by Steiner (l.c.); the males, on the other hand, are usually straight. Adults of both sexes are rather sluggish in movement but the males are more motile than the females.

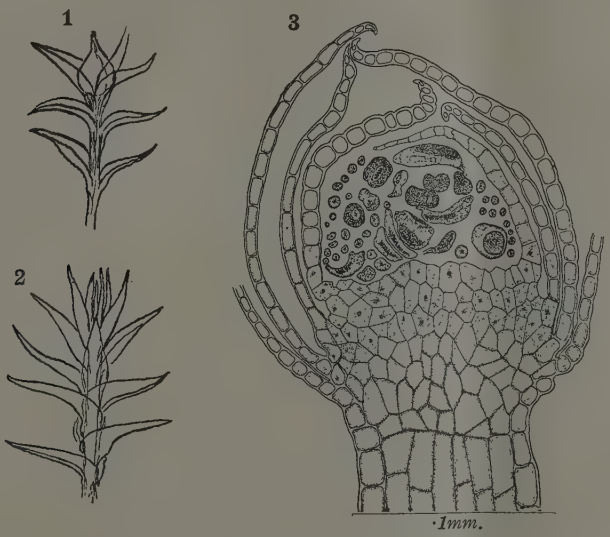
Morphology of the Parasite.

Dimensions: *Female,* length, 0·7 mm. to 0·9 mm.; a = 14·6 – 19·3; b = 5·2 – 7·3; c = 12·2 – 14·3; V = 78% – 83% (7 measured). *Male,* length, 0·63 mm. to 0·71 mm.; a = 24 – 27·6; b = 4 – 4·5; c = 9·2 – 10·9 (5 measured), eggs, 70μ long by 35μ wide, spicules, 25μ to 26μ long.

As the dimensions show the adults are remarkably short, the females particularly being very stout. The body tapers a little anteriorly in both sexes to the head end and posteriorly to the sharply pointed tail. The cuticle carries fine transverse striations and a lateral field is present on either side about $\frac{1}{3}$ as wide as the body. The writer has failed to find longitudinal markings on the lateral fields as figured by Steiner on those of *A. askenasyi.*

Head slightly offset by a fine constriction, rather flat and with rounded sides. Head papillae not seen. Head framework typical and not very strongly built. Mouth spear about 10μ long and of the usual structure; base with distinct rounded knobs. Oesophagus with an almost cylindrical pre-corpus which is not constricted immediately in front of the median corpus or muscular bulb. Latter rather narrow and with delicate crescentic thickenings of the lumen at the centre. Isthmus about same length as pre-corpus and then swelling out into the terminal glandular region consisting of the oesophageal gland cells. This region is not sharply marked off from the beginning of the intestine but is rather lobed and occasionally a little irregular in outline where it wraps round the tapering commencement of the intestine. The nucleus of what is probably the dorsal oesophageal gland cell is large and prominent; the nuclei of the sub-ventral gland cells are inconspicuous. The opening of the dorsal gland cell into the lumen of the oesophagus occurs close behind the spear base. The excretory pore lies ventral to the terminal region of the oesophagus.

Female. The body tapers gradually from the vulva backwards and the tail end, which is more or less conical in shape, has a very sharp point. The vulva has rather prominent, rounded lips and lies rather far back on the ventral surface at 78% to 83% of the body length from the anterior end. In the case of *A. askenasyi* its position, according to Steiner (l.c.), is 73% to 78% of the body length. The gonad is



Pottia bryoides Mitt.

Figs. 1 and 2.—Freehand sketches of two shoots of the moss, *Pottia bryoides*; a gall-bearing shoot above and a healthy shoot below × 12, approx.

Fig. 3.—Longitudinal median section of galled shoot of *P. bryoides* showing structure of gall in the cavity of which are various sectionised portions of the parasite. Note the granular tissues forming the floor of the gall cavity × 360.

single and directed forward and the ovary is reflexed twice towards its anterior end so that its rounded tip often lies over the terminal region of the oesophagus (fig 4.) A remarkable feature of the ovary is the large nuclei of the cells forming its covering membrane. It gradually increases in width posteriorly and is finally constricted at its junction with the swollen anterior end of the uterus which here forms a receptaculum seminis. The uterus wall is composed of large polygonal cells containing vacuolate protoplasm. There is a distinct post-vulval uterine sac.

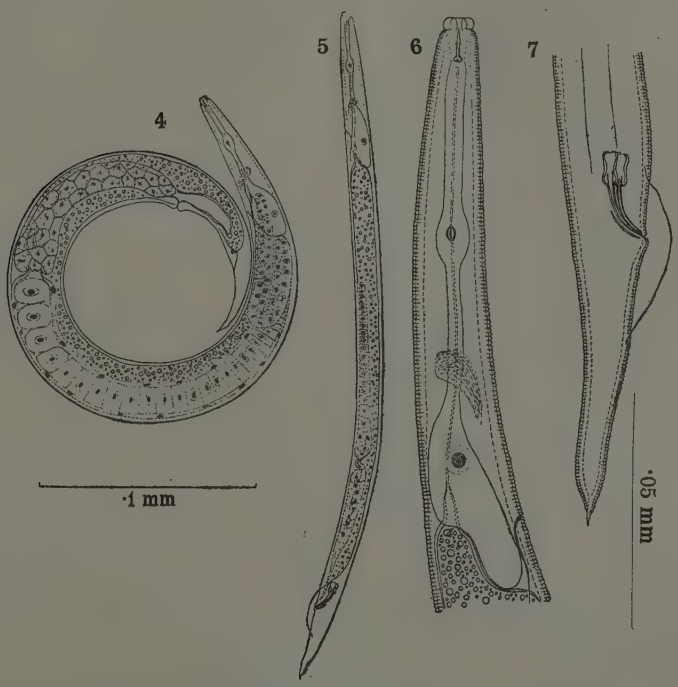
Male. The testis is single, anterior and outstretched and its front end is located at about the anterior third of the intestine from the end of the oesophagus. The broader posterior end of the testis is separated from the vas deferens by a short constricted duct and the vas deferens itself ends in a rather long, tapering ejaculatory duct. The spicules are slightly arcuate and shaped as shown in fig. 7. They rather closely resemble those of *A. dipsaci* in structure; each consisting of an expanded anterior third and a tapering shaft. The gubernaculum is simple, linear and about $\frac{1}{3}$ as long as the spicules. The characteristic bursa consists of two lateral alae which arise at about the level of the heads of the spicules, are widest opposite the cloacal aperture and are inserted a little less than halfway between the latter and the tip of the tail which ends in a steeply tapering and sharply pointed tip. The caudal alae in being inserted about half-way down the tail have the same disposition as those of *A. askenasyi* as figured by both Bütschli and Steiner.

Systematics.

In view of the fact that comparatively little is known about the anatomy of the nematodes causing galls on mosses and because of the confusion existing for so many years between *A. askenasyi* and *A. dipsaci,* some discussion on the systematic position of the new form is called for. In general anatomical features it resembles *A. askenasyi,* as figured by Steiner (l.c.) but it also presents certain clearly marked differences. Thus in size it is much shorter than the lengths given for *A. askenasyi* for which Bütschli gave 1·7 mm. for the female and 1·4 mm. for the male from *Hypnum cupressiforme* and Steiner 0·98 mm. to 1·2 mm. for the female and 0·92 mm. to 1·2 mm. for the male from *Thuidium delicatulum.* The longest female found by the writer was only 0·9 mm. long and all the other specimens were shorter than this. The males, too, are correspondingly shorter than those of *A. askenasyi;* the longest one found being only 0·71 mm. long. In the length of the mouth spear, approximately 10μ long and the position of the excretory pore ventral to the glandular oesophageal swelling, the new form agrees with *A. askenasyi.* In the somewhat lobed and irregular shape of the terminal region of the oesophagus which wraps round the beginning of the intestine for a short distance it differs from the form figured by Steiner.

A distinct difference from *A. askenasyi* is presented by the doubly reflexed character of the anterior end of the ovary which in *A. askenasyi* is not reflexed at all but is outstretched. Three other differences may

also be noted namely, the smaller size of the eggs which are 70μ long by 35μ wide whereas in *A. askenasyi* they are 80-100μ long by 36-41μ wide, the smaller spicules, 25-26μ long as compared with 30μ in *A. askenasyi* and the apparent absence of longitudinal markings on the lateral fields.



Anguillulina brenani n. sp.

Figs. 4 and 5.—Adult female and male respectively of *A. brenani* to show general shape and structure.

Figs. 6 and 7.—Oesophageal region and tail of male under high magnification in side view.

These differences together are sufficient, in the writer's opinion, to warrant the creation of a new species for the nematodes described in this paper and they are accordingly named *Anguillulina brenani* n.sp. in honour of Mr. J. P. M. Brenan who discovered the galls and the nematodes in them.

Host. Pottia bryoides Mitt., a moss belonging to the order Tortulaceae.

Gall Structure.

As already pointed out above the galls are pyriform in shape and terminally situated on main or branch shoots. Careful dissection with needles under the microscope showed that each gall is made up of a series of leaves the inner ones of which form a sort of dome-like covering which encloses a cavity the base of which is formed by the end of the shoot. This characteristic structure was further elucidated by the preparation of longitudinal sections of gall-bearing shoots. These were fixed in formol-alcohol and after processing through dioxan were embedded in paraffin. Longitudinal sections 10μ thick were cut on the microtome and were stained in Delafield's haematoxylin. From an examination of these sections it can be seen that the presence of the parasite has the effect of causing the innermost of the terminal leaves of a shoot to arch inwards so that their tips overlap each other. The hollow space thus formed below them and the top of the stem forms the gall cavity in which the parasites live. The outermost leaves of a gall remain pointed and form the narrower gall tip. Young galls are uniformly green but older ones turn deep brown and there appears to be a considerable thickening of the cell walls especially of those leaves forming the outer layers of the gall.

Another noticeable feature of the older galls is the granular character of the cells forming the floor of the gall cavity. This is shown in fig. 3 where the tissues to a depth of 3 or 4 cells have rather large nuclei and distinctly granular protoplasm. Doubtless this tissue corresponds to the " nutritive zone " found in so many other galls of nematode and insect origin in which the tissues immediately surrounding the gall cavity have a highly granular character and no doubt serve as a source of nourishment to the parasite.

Nothing is known, so far, about the life history of the parasite and further observations are necessary to determine, among other points requiring elucidation, which particular larval stage functions as the infective stage.

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A Helminthological Survey in Northern Rhodesia.

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The results of an investigation into the nature, incidence and distribution of human helminthic infections in Northern Rhodesia are described in the present report. This inquiry was undertaken with a view to estimating the importance of these infections in relation to the health of the native population, hence indirectly to that of the European community; and also for the purpose of studying the conditions which influence, adversely or favourably, the propagation and spread of helminth parasites in Northern Rhodesia. The survey was of the same duration of about ten months and followed the same general scheme as that of Blackie (1931) in Southern Rhodesia, and consisted of two parts, a period of laboratory study and a period of touring in the native reserves. It differs from the S. Rhodesia survey in that relatively more attention and time were allotted to the latter category—a procedure necessitated by the fact that N. Rhodesia has an area almost twice as great as that of its southern counterpart and communications which are more susceptible to interference by seasonal and climatic changes. In planning the itinerary of the survey the choice lay between covering a large area in a superficial manner or a smaller area more thoroughly. The latter course was deemed to be preferable and more likely to yield results of value, and although it proved to be slow and resulted in the survey of only about one-fifth of the total territory, the decision was probably justified.

The position with regard to the current knowledge in recent years of helminthic infections in Northern Rhodesia and the increasing awareness of the Medical Authorities as to their incidence and possible importance in the health and economic life of the country are indicated in the Medical Reports on Health and Sanitary Conditions for the years 1935-1940. Although no accurate information was available concerning their incidence and distribution up to that period, helminthic infections were being regarded as more prevalent in the country than was previously realised. Of the more common human helminths that had been reported from time to time chief interest centred about bilharzia and hookworm. Infestations with the latter form were believed to be widespread, common and generally light in character but it was admitted that its economic importance was an unknown quantity.

Bilharziasis, in both the vesicular and intestinal form, was being reported with increasing frequency, and interest was maintained in its peculiarly uneven distribution, its sub-clinical character and the problems concerning its accurate diagnosis.

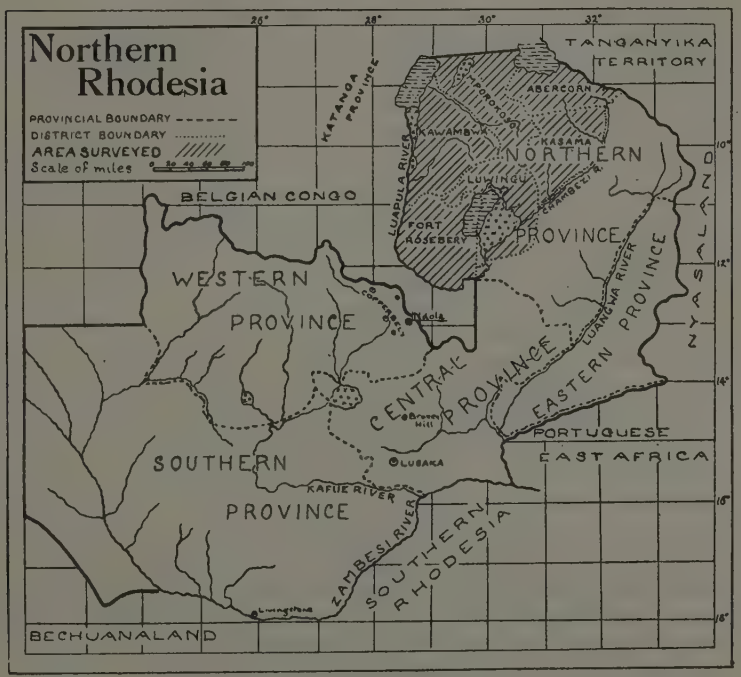
Of the other human helminthic infections known to be indigenous in the country, *A. lumbricoides* was thought to be widespread and probably of universal occurrence in the Bangweulu swamps. Less frequently recorded were *Trichuris trichiura, Enterobius vermicularis, Strongyloides stercoralis, Taenia solium, T. saginata* and *Hymenolepis nana*. Of the filarial parasites only *A. perstans* was known. *Wuchereria bancrofti* had not been definitely recorded and the cases of elephantiasis in certain districts, also known as " Serenje leg " and " Feira leg," were believed to be non-filarial in origin.

Physiography.

Northern Rhodesia lies between longitudes 22° E. and 34° E. and between latitudes 8° S. and 18° S. It has an area of about 290,000 sq. miles and is roughly divided by a tongue of Belgian territory intruding into it from the north-west to a depth of about 100 miles, into two unequal parts; a north-eastern region of high elevation which forms the eastern extremity of the Congo River watershed, and a western region of greater area and lower altitude which includes part of the Zambesi River basin. The European population in the year 1940 was approximately 13,000 and the African population approximately 1,400,000.

The north-eastern region, or Great Plateau as it is also called, in which the present survey was carried out, is bounded on the north by Tanganyika Territory, Lake Tanganyika and Belgian Congo; to the west by Lake Mweru and Belgian Congo; to the east by Nyasaland and to the south by Portuguese East Africa. It has an altitude of some 4,000-6,000 ft. above sea level and slopes mainly from the north-east to south-west, but there is a secondary inward slope to a large central depression which forms the lake and swamps of Bangweulu, while to the east the great valley of the Luangwa River cuts obliquely into the plateau surface. From its highest elevations in the north-east the plateau falls away steeply to Lakes Tanganyika and Rukwa but extends for some distance northwards between them. Between Lake Tanganyika and L. Mweru the fall is more gradual, to the curious dead lake, Mweru Wantipa, and to L. Mweru itself, both of which are above the level of L. Tanganyika. The western boundary is marked by the winding river Luapula, which starts on its long and devious course as the Chambezi River in the high land to the north-east, from which ample watershed

it flows through the central depression, turns north again in a shallow valley and with increasing magnitude finally passes through a widening belt of swamps to enter Lake Mweru. To the east the elongated Muchinga mountain range separates the Luangwa River, a tributary of the Zambesi from the Chambezi River which is the most easterly origin of the Congo River.



Map 1. Showing area surveyed.

The basic structure of the plateau consists of rocks of Archaean origin, schists, quartzites and limestones, much folded and metamorphosed, through which granite constantly intrudes. The long Muchinga escarpment is composed mainly of granite and schists; these rocks are a feature of the eastern half of the plateau. In the western half, or roughly to the west and south-west of a line drawn from Abercorn to the Lukulu-Chambezi confluence, sandstones of the Katanga system are superimposed on the igneous base. The surface of this western

half, in contrast to that of the east is characterized by easy undulating country, through which in a few places portions of the igneous base appear, notably in the Luapula escarpment to the north-west and at Chiengi on L. Mweru, and also to the south-east of Fort Rosebery. An elevated area at Kasama represents an easterly extension of the sedimentary series.

The predominant feature in the vegetation of the plateau is a type of thin forest of almost monotonously uniform appearance which occurs everywhere except in the swamps and on some of the high hills. Near Lake Tanganyika there is tall tropical forest, but elsewhere this type is poorly represented and is only seen as isolated relics in a few places.

Topography of the Area Surveyed.

The area surveyed comprises the north-western half of the plateau, or, that region embraced by the course of the Chambezi and Luapula Rivers to the east, south and west, and bounded on the north by Lake Mweru, Mweru Wantipa and Lake Tanganyika. The six districts Fort Rosebery, Luwingu, Kawambwa, Mporokoso, Abercorn and Kasama lie within these boundaries. The following data on subjects relevant to the survey will relate mainly to this area.

Climate.—The single rainy season lasts approximately from November to March, its duration and rainfall varying in different topographical regions. The highest recorded rainfalls are from the centre of the plateau on Lake Bangweulu where the mean annual figure for six years was 57.18″, at Chilubi Island. A region of low rainfall is indicated to the north-west by the low figures of 31.41″ at Kafulwe and 36″ at Chiengi on L. Mweru and by the type of vegetation occurring in the adjacent lowlands. There appears to be no correlation between rainfall and altitude, as indicated by the average annual rainfall figures for the period 1938/39 to 1943/44 from ten stations on the plateau which are as follows: Kafulwe, 31.41″ (2-yearly mean) (3,000′-3,500′); Johnston Falls 41.10″ (3,000′-3,500′); Chilubi Island, 57.18″ (3,700′); Fort Rosebery, 40.05″ (4,000′-4,500′); Kawambwa, 51.35″ (4,000′-4,500′); Kasama, 51.21″ (4,000′-4,500′); Mporokoso, 48.25″ (4,500′-5,000′); Luwingu, 52.63″ (4,500′-5,000′); Abercorn, 45.94″ (4,500′-5,000′) and Lunzuwa, 38.86″ (5-yearly mean) (5,000′-5,500′).

Temperature data for the plateau are somewhat limited. The mean maximum lies between 82° and 86° F; the mean minimum between 54° and 58° F. The high country to the north-east has cool conditions at certain times and sharp frosts may occur in places, whilst at Chiengi in the west the mean minimum temperature is as high as 66.4° F. It

would seem that a more consistently tropical climate exists there and in the lower Luapula valley.

Population.—The population of the Chambezi-Luapula area consists of less than one hundred Europeans and some 340 thousand Africans. The European community is composed of Government officials, missionaries, merchants and farmers. The native population embodies various tribes of Bantu origin whose present day definitions are not always clear-cut since their history up to the end of the last century has been much complicated by constant movement, intermingling and warfare. Of the fifteen tribes or tribal offshoots which occupy this area, the most important are the Awemba, a war-like people who came from the south in the early 18th century and succeeded in dominating the previously established tribes. Their influence is reflected in the number of tribes, some of them from the Congo, whose dialects resemble the language of the Awemba. The average population density is about 7 per square mile, but this is magnified about fifty times in the lower Luapula valley and in parts of the Bangweulu region, notably Chilubi Island and on the western shore of the Lake.

Agriculture and Foodstuffs.—The chief crops grown are finger-millet, cassava, Kaffir-corn and, to the east of the plateau, maize. Differing types of agricultural methods are used, which vary according to the nature of the crop and to the topography of the country. The most widely used method is the tree-cutting Chitemene system, or local variants of it. In this system the relatively poor quality of the soil is enriched by the addition of wood-ash which is obtained by lopping off the upper branches of trees from a large area of woodland, dragging these to the smaller area to be cultivated where they are burned *in situ*. This form of cultivation is employed for finger millet which is a crop suitable for higher altitudes and is the staple in Mporokoso, Abercorn and Kasama districts. It involves the shifting of the village site when successive crops and clearings have encroached on the woodland to such an extent as to make the distance between garden and village too great; and, what is probably more important from the aspect of helminth transmission, it means not only that the garden is more or less distant from the village for the greater part of the latter's duration but also that the garden site varies in its location from year to year. During the dry season, June to October, the work on the gardens consists of tree-cutting and preparing the ground while the wet season, November to May, is occupied with sowing and harvesting. Of this work the tree-cutting is the exclusive duty of the men. Hoeing is done only by the women.

Cassava, the other principal crop grown in the Chambezi-Luapula area, is of increasing importance and is widely used in the low-lying regions of the three districts Fort Rosebery, Luwingu and Kawambwa. Its cultivation does not necessarily require the ash-supply so that tree-burning and frequent changes of village sites are not involved. Village settlements in these three districts are for the most part of fairly permanent or semi-permanent duration. Another point of difference between cassava and finger-millet cultivation, again possibly one of importance in the epidemiology of certain kinds of helminthic infections, lies in the fact that cassava gardens are usually in close proximity to the villages.

A considerable variety of subsidiary foodstuffs is also cultivated by the plateau native but in times of famine or food scarcity he may have to supplement his diet from natural sources and fall back upon wild roots and fruits, insects, caterpillars and snails. Fish is an important foodstuff and means of livelihood for a large proportion of the population of the Luapula valley and the Bangweulu area.

Livestock is not of much importance in the agricultural economy of the plateau and with the exception of Abercorn, Isoka and Fort Jameson districts, cattle are practically non-existent. Tsetse fly and tribal tradition—the Chiwemba speaking tribes are mainly non-pastoral —are chiefly responsible for this scarcity which in turn accounts for a very low incidence of *Taenia* infestations amongst these tribes. Goats and poultry are ubiquitous but sheep and pigs are very rarely seen.

Wild game being plentiful is hunted or trapped by the native who values very highly a supplementary meat diet of any kind and is not averse to consuming any part or organ—a fact which should receive special consideration during routine laboratory diagnosis of helminth infections since it is liable to give rise to pseudo-infections.

Methods Employed in the Survey.

The procedure adopted in the survey was to visit each district in turn and to examine natives in their villages in different topographical regions. Having chosen a suitable Location a camp and field laboratory were set up from which villages within about a 10 miles radius were investigated. After a representative number had been examined, the camp and laboratory were moved to a new Location where the process was repeated. The position and number of the Locations, which are indicated on Map II were limited to some extent by transport facilities, since only those areas served by motor road or water route could be

visited; time-consuming foot treks to less accessible places could not be undertaken. Nevertheless a considerable variety of types of country and population were attainable and this limitation did not prove to be a very serious one; but towards the close of the survey freedom of movement to the outlying parts of Abercorn and Kasama districts was somewhat hampered by the onset of the rainy season.

Technique of collecting, preparing and examining specimens.—For the collection of faeces and urine specimens in the villages, each individual was supplied with two cork-stoppered 3″ x 1″ glass tubes one of which was provided with an aluminium spoon, and a slip of paper bearing name, age, sex and name of the village. Individuals were unselected from any clinical standpoint but were representative in so far as was possible with regard to age and sex. In the tabulated results the categories " children " and " adults " are used and are definable as up to 15 years and over 15 years respectively.

In preparing the specimens a standardised concentration method was adhered to which is essentially the same as that employed by Blackie (1931). Briefly, this is as follows: to the faecal specimen, which varied in size from about 1cc. to 5cc., normal saline was added until the container was 3/4 full and an emulsion was made by thoroughly mixing faeces and saline with a glass rod. The emulsion was then passed through a sieve into a Petri dish to remove coarse debris. (A small bakelite tea-strainer proved admirable for this purpose and had the advantage over wire gauze of being easy to clean.) The strained emulsion was then returned to a clean 3″ x 1″ tube, allowed to stand for 30 minutes after which time the supernatant fluid was poured off leaving only the egg-bearing sediment. To this was added enough normal saline to make up a volume about equal to that of a centrifuge tube. After mixing thoroughly it was decanted into a centrifuge tube and spun for 30 seconds. A few drops were then drawn off from the bottom of the tube with a pipette, transferred to a slide, covered with a cover-slip and examined under a magnification of x 150. The urine specimen was allowed to stand in its container for 30 minutes after which time the supernatant fluid was poured off leaving about ½ inch in the bottom. This was transferred to a centrifuge tube, spun for 30 seconds and then examined in the same way as the faecal specimen.

A restriction on the collecting of blood specimens in the villages imposed a limit to the enquiry into the incidence of filarial infections and information in this direction could only be obtained in the Government or Mission hospitals which were encountered en route. For this purpose

thick films were taken from in-patients at night and during the day. These were dried, dehaemoglobinized and stained with Giemsa either by the slow cold method or by the rapid method using heat. For the diagnosis of possible *Onchocerca* infections the " skin-snip " method was used. This consists of removing from the forearm, with a sharp razor blade, a very thin sliver of skin, 0.1 to 0.3 mm. in thickness and about 25 square millimetres in area. This is placed on a microscope slide, covered with a drop or two of normal saline and examined directly. The microfilariae of *O. volvulus* if present in the skin escape rapidly and are easily detected swimming actively in the saline under a magnification of x 150.

Results of the Survey.

In this section each of the six administrative districts concerned in the survey is treated separately and the incidence of helminthic infections in all of its Locations is summarised in tabular form. Since however a district is not a topographical unit, although it is a geographical one, the relationship between helminthic infections and topography in the area as a whole is depicted in a different manner in a later section by a series of maps each illustrating the distribution and incidence of the principal infections. In these maps the infection rates in the Locations constitute the guiding principle in estimating the trend of infections and district boundaries are disregarded.

Fort Rosebery District.

This district is situated in the loop formed by the Luapula River in its course from Lake Bangweulu to where it turns north to Lake Mweru. It is relatively low-lying, 3,500′-4,000′, with an area of about 8,490 sq. miles and a population of about 75,000 which is very unevenly distributed, for more than half this number are crowded into a narrow belt on the western shore of L. Bangweulu and also to the south of it. The distribution of the remainder tends to follow the principal waterways, namely the Luapula River and its few larger tributaries; large areas of this district are thus virtually uninhabited.

Population samples were examined for helminthic infections in four different Locations.

Location One. (Kapalala.)

The main route from the Western Province to the Northern Province through the Belgian Congo pedicule crosses the Luapula by ferry at this point. The river here is wide and deep and valleyless and its only tributaries are small streamlets which may fail during the dry season or if not, become mere trickles which tend to be lost in level treeless

swampy areas (dambos) and reappear lower down. The north bank is dotted with villages most of which are within easy distance of the river and draw their water from it but may also employ other sources such as wells, lagoons, inlets and small streams. The small streams are the most favoured habitat of aquatic snails of the genera *Physopsis, Bulinus, Biomphalaria* and *Lymnaea*. The lagoons and inlets harbour them also but they were never found in the wells or in the Luapula itself. (A " well " is merely a water-hole of shallow depth and a few feet to a few yards in diameter. It is usually kept free of vegetation.)

Specimens of faeces and urine from 304 natives were examined in this Location, representing 17 villages having a range of 16 miles along the north bank of the Luapula River and not more than about 2 miles distant from it. The people belong to the Kawendi tribe whose villages are permanent or semi-permanent. The chief crops grown are cassava and Kaffir corn. The helminth infections found were hookworm (62.8%), *Strongyloides* spp. (10.2%), *Ascaris lumbricoides* (1%), *Enterobius vermicularis* (2%), *Trichostrongylus* sp. (1 case), *Schistosoma haematobium* (30%), and *S. mansoni* (8.8%). Analysis of these results reveals some points of interest, especially with regard to the bilharzia infections. Thus, the incidence of *S. haematobium* is not only greater than that of *S. mansoni* but was distributed more evenly throughout the Location. In the case of *S. mansoni* only those villages in the eastern or upstream part of the Location were affected to any extent, where the incidence was 14%, as against 1% in the western part. Another point of divergence in the characters of these two kinds of infections was exhibited in the infection rates in children and adults. Thus, the eggs of *S. haematobium* were found in the urine of 52% of all the children examined and only in 15% of the adults. This difference is not only statistically significant in the present instance but was found to be consistently significant in every other Location where the infection was endemic. It will be discussed in greater detail in a subsequent section. *S. mansoni* on the other hand was present in adults and in children in about equal numbers, namely 8.1% and 9.5% respectively.

In view of the current belief, derived from Tour Reports of Medical Officers in the Fort Rosebery district, that Kapalala represents the upstream limit to bilharzia distribution along the Luapula River, two villages (Thomashi's and Kalebwe) situated 30 miles upstream were sampled. Nine cases (19%) of *S. haematobium* were found and four (6.5%) of *S. mansoni*. These figures are considerably lower than the averages for Kapalala and may possibly be associated with the greater

proximity of these villages to the Bangweulu region where bilharziasis is so conspicuously absent. The hookworm rates (77%) as in the case of the other riverine villages is high.

A topographical contrast to the riverine villages was afforded by an isolated " inland " village (Macheto) about 12 miles north of Kapalala on the Fort Rosebery Road, which is dependent for its water supply on wells in the dry season, and on a small impermanent stream. Here the hookworm rate was low (33%) and only one case of bilharzia, *S. haematobium,* was found in the 21 people examined.

Aquatic molluscs were searched for throughout the Location; the species taken and their localities are listed elsewhere. Potential bilharzia vectors in particular were collected and examined for natural infections but no conclusive evidence was obtained on these lines nor was the number of localities in which they were taken representative enough to provide an explanation for the uneven distribution of the adult infections in the villages.

Location Two. (Lwela River.)

This tributary of the Luapula River is well populated with villages along its whole length. In the present Location, where ten villages were sampled for helminthic infections, the country is not very flat and the small streams entering the Lwela River are more numerous and more permanent than those of the Luapula in Location One. The people belong to the Ushi tribe and grow Kaffir corn, finger-millet and cassava. The type of agriculture here is somewhat variable as it is on the line of transition from the Southern Chitemene Contact, but the villages for the most part are fairly permanent.

The results of over 200 examinations in this Location showed considerable differences in helminthic infections from those seen at Kapalala. Both the hookworm and *Strongyloides* incidences are much lower (40% and 4.1% respectively) but the *S. haematobium* incidence is higher (62%) and 80% of the children were found infected. Only one case of *S. mansoni* was seen, although both *Biomphalaria pfeifferi* and *B. tetragonostoma* occur in the vicinity. Other helminth eggs seen were *Trichostrongylus* sp. (two cases), *Enterobius* (one case), *Hymenolepis diminuta* (one case) and Spirurids (two cases). In addition there was a single case of an adult male who was passing very small trematode eggs containing an apparently viable miracidium. These may have been from one of the Opisthorchid group of parasites, but it is uncertain whether or no it was merely a case of pseudo-parasitism since a re-examination proved negative.

Location Three. (Fort Rosebery Boma.)

The town or boma of Fort Rosebery is situated on the Mansa River which flows due west into the Luapula. Of smaller dimensions than the Lwela River it is well served by small tributaries, one of which—the Kanwabateni—was found to be heavily stocked with *Physopsis globosa*.

The natives belong to the Ushi tribe who grow cassava and finger-millet, employing the Western Chitemene system. Villages in the vicinity of the town probably remain stationary for a number of years and do not move long distances.

Two villages situated not far from the town together with the native compound housing Government clerks, staff and their families comprise the subject matter of this Location.

Of the 80 villagers examined 34% had hookworm and 42.5% *S. haematobium* (i.e. adults 25% and children 57%). No *S. mansoni* was seen. The *Strongyloides* incidence of 5% corresponded with the low incidence of hookworm and it was interesting to note throughout the survey the parallel course followed by these two infections.

In 30 inhabitants of the Government native compound all the helminth infections were on a lower scale, with hookworm (13%), *Strongyloides* (3.3%) and *S. haematobium* (10%).

Ten Europeans who had been resident in the town for at least a year were examined for helminth infections. All were negative.

Unidentified tapeworm eggs of the *Hymenolepis* type were seen on one occasion in the faeces of an adult from one of the villages. They measured about 68μ × 57μ and possessed three envelopes or shells. Polar filaments were absent and the embryonic hooklets measured 12-15μ. Further investigation of this infection or pseudo-infection could not be followed up as the carrier could not be traced.

Location Four. (Mwamfuli.)

This Location provides some marked topographical contrasts to the previous ones; thus, it is on the shore of Lake Bangweulu and the only water courses are infrequent small streams of the same category as those at Kapalala. The surrounding country is some 30 to 50 feet above lake level and the shore slopes rather steeply to the water, in some places resembling a cliff. It is inhabited by the Kawendi who grow cassava, employing the Lake Basin System. Snails of the family Bulinidae were found in two of the small streams entering the lake. In the lake itself only *Lanistes ovum* was seen.

Population samples (154) were examined from four villages, two of

which are near the lake, one two miles inland and one six miles inland. Two points of interest emerge from these; firstly, the high hookworm (63%) and *Strongyloides* (9.7%) incidences which resemble those of Kapalala rather than those of the less distant Locations two and three; secondly, the almost dramatic fall of the bilharzia rate to 3.2% in *S. haematobium*. (A single case only of *S. mansoni* was seen.) The *Ascaris* incidence of 8.4% was the highest yet encountered and is illustrative of the prevalence of this infection in the lake region.

TABLE I.

Helminthic Infections in Natives of Fort Rosebery District.

	Adults		Children		
	Male	Female	Male	Female	Totals.
Number examined for intestinal helminths	148	285	203	150	786
Hookworm	50%	53·7%	53·7%	48%	51·9%
Ascaris lumbricoides	1·35%	1·4%	3·0%	2·6%	2·0%
Trichostrongylus sp. ...	0%	0·7%	0·5%	0%	0·38%
Strongyloides spp. ...	6·7%	7·7%	8·8%	6·6%	7·6%
Enterobius vermicularis	0%	2·1%	0·5%	2·6%	1·4%
Schistosoma mansoni ...	2·7%	4·2%	3·9%	3·3%	3·6%
Hymenolepis diminuta...	0%	0%	0·5%	0%	0·125
Unidentified cestode ...	—	—	—	1 case	—
Number examined for urinary helminths ...	148	284	203	147	782
Schistosoma haematobium	20·2%	22·4%	55·1%	42·0%	34·2%

Summary of Helminthic infections in Fort Rosebery District.

The results of faeces and urine examinations in four Locations in this district are summarised in Table I. The outstanding infections are seen to be hookworm and vesicular schistosomiasis, but their incidences in different Locations are somewhat variable. It would appear that hookworm is more prevalent in villages bordering the Luapula River itself than in those situated along its larger tributaries, the Lwela River and Mansa River. The western shore of Lake Bangweulu is also highly endemic and villages even 2 to 6 miles inland from the lake have a high infection rate. With regard to *S. haematobium* the heaviest infection rate is on the Lwela River but the Mansa River and Luapula River also show high incidences. From the uniformity of its occurrence along the 16 mile riverine stretch in Location One it is probable that this infection is endemic along the Rhodesian bank of the Luapula River from

Kapalala to Johnston Falls in Kawambwa District. Upstream from Kapalala it appears to diminish in intensity as the Lake and Swamp region is approached. The presumptive areas of high endemicity of hookworm and *S. haematobium* are indicated in Maps III and VI.

Aquatic Molluscs from Fort Rosebery District.

Species.	*Locality.*
Biomphalaria pfeifferi (Krs.)	Nkulumashiba R. (Trib. of Lwela R.) (2)
B. tetragonostoma (Germain).	Lagoon, Kapalala (1). Lobe R. (Trib. of Luapula R.) (1). Nkulumashiba R. (2).
Planorbis costulatus Krs.	Mansa R. (3).
*Lymnaea natalensis** Krs. (resembling *L. humerosa* Mts.)	Lobe R. (1).
*L. caillaudi** Bgt. var. *undussumae* Mts.	Nkulumashiba R. (2), Lobe R. (1).
*Physopsis africana** Krs. (apparently).	Chimana R., Mwamfuli (4).
*P. africana** Krs. (Nearest).	Chipwala R. (Trib. of Lwela R. (2).
*P. globosa** Morel. (Nearest).	Kanwabateni R. (Trib. of Mansa R.) (3).
*P. africana** (?*Bulinus natalensis*)	Lobe R. (1).
*Bulinus natalensis** (Küst).	Kasamba R., Mwamfuli (4).
*B. natalensis** (Küst). (Approaching *Physopsis globosa* Morel).	Nkulumashiba R. (2).
*B. natalensis** (Küst). (Very like *B. depressus* Haas).	Lagoon, Kapalala (1). Lunuka R. Kapalala (1).
Cleopatra hargeri Smith.	Mansa R. (3). Lunuka R., (1).
Lanistes ovum Troschel.	Lake Bangweulu (4).
Viviparus sp. juv.	Kasamba R. (4).

* Concerning these genera, Major Conolly, who identified all the snails listed in this report, remarks :

(i) " It is practically impossible to distinguish shells of *Physopsis globosa* Morel. from *Bulinus natalensis* (Küst). The angulation of the columella and rimation of its margin vary so enormously in shells of almost every small assemblage that most could apparently be assigned with small hesitation to either species, which applies to the majority of all specimens of these genera found throughout Rhodesia.

(ii) " Judging from Martin's figures *Lymnaea humerosa* and *undussumae* represent an extremely slender form of *L. caillaudi* Bgt. and are well represented in many localities, more especially in the Northern Province, while resuming the less slender, more usual form towards the South."

All the other helminthic infections occur to a much lesser degree, but certain villages in the Kapalala Location have a high infection rate with *S. mansoni*.

Aquatic molluscs were collected in the district during the period June to August and are listed on page 123. The numbers in parentheses refer to the Locations concerned.

LUWINGU DISTRICT.

Part of Lake Bangweulu and most of its adjoining swamps occupy a very large area in this district whose watery character is further enhanced by the high annual rainfall which occurs in its lowest as well as in its highest regions. A large proportion of the native population of some 55,000 inhabits the lake and swamp area and there is over-population in some places. Cassava is the chief crop grown but towards the northern part of the district there is a transition to millet and the Chitemene system. Five Locations were sampled, each having different topographical characteristics.

Location Five. (Nsombo.)

Like the previous one (Mwamfuli) this is a lake shore Location which differs in that the shore slopes gradually down to the water. Rivers or streams flowing towards the lake tend to be lost hereabouts in an extensive swampy foreshore.

One hundred and twenty-five individuals representing ten foreshore villages were examined for intestinal and urinary helminths. The high incidence of hookworm (73.6%) and *Strongyloides* (24.8%) were not unexpected in view of the local conditions but the low *Ascaris* incidence of 4% is not easy to explain. The truth of the bilharzia-free Bangweulu legend was again confirmed, for only two cases, both rather doubtful, were encountered. Of considerable interest in this Location was the finding of viable eggs of an unidentified trematode in the stool of an adult female. These were large (120μ x 68μ) pale yellow and thin-shelled with an operculum of 25μ in diameter. A repeat examination was positive and strong efforts were made to persuade the carrier to submit to anthelmintic treatment at the hospital on Chilubi Island with the object of recovering the adult worms, but unfortunately without success.

The only snails found were *Lanistes* and *Viviparus*. A form of *Bulinus natalensis* however was once recorded from here by Haas (1936) in " a canal from Nsombo to the lake ".

Location Six. (Chilubi and Chisi Islands.)

Chilubi Island is situated near the eastern shore of Lake Bangweulu. The " shore " in this case is merely the edge of a vast swamp which extends over about 2,500 sq. miles to the north, east and south of the lake. The island has an area of 35-40 sq. miles with an irregular coast line about 40 miles long. It is flat, thinly covered with vegetation, riverless and waterless, hence the population of some 10,000 is concentrated along the periphery and must represent a density far greater than the theoretical average of 250 per sq. mile. Chisi Island, a few miles to the north west, is smaller than Chilubi, but is similar to it in most other respects. It is included as part of this Location for topographical reasons although in reality it lies in Fort Rosebery District.

Eighteen villages on Chilubi and three on Chisi were sampled. The helminth infection rates are essentially similar in both places and accordingly the figures are combined, with the following result. In the 132 individuals examined for intestinal and urinary helminths there was hookworm (72.7%), *Strongyloides* (5.3%), *Ascaris* (15.9%), *Trichuris* (3 cases) and *Enterobius* (2 cases). Bilharzia* appears to be completely absent in this type of lake Location and no potential snail vectors were found; the non-carriers *Lanistes* and *Viviparus* were the only snails collected.

The main difference between the helminth incidence in this Location (Lake Island) and that of the previous one (Lake Shore) is exhibited by *Ascaris* and *Strongyloides*. The higher incidence of *Ascaris* on the Lake Islands might be attributed to the congested living conditions resulting from over-population. The higher incidence of *Strongyloides* in the Lake Shore Location might possibly be explained by the more favourable environment provided by the swampy foreshore for the free-living development of this parasite.

Location Seven. (Nsalushi.)

The Bangweulu Swamp is habitable mainly on a limited number of low-lying outcrops of firm ground, the " sand-bank islands ", which are usually densely populated and are seasonally isolated by the rising water level. A typical example of one of these is Nsalushi on the main channel of the Chambezi River. It is inhabited by the Unga tribe who like most of the swamp islanders grow cassava as the principal crop. Faeces and urine specimens from 84 individuals, representing eight villages, were examined and showed the following helminth

* Bilharzia—species not stated—was recorded as being common in Chisi islanders in 1936 in a Tour Report from the Health Department.

infections: hookworm (74%), *Strongyloides* (32%), *Ascaris* (62%), *Trichuris* (6%), *Enterobius* (4%), and *S. haematobium* (2 cases). Conditions here combine the congestion of Chilubi and the swampy environment of Nsombo, which may account for the high incidence of both *Ascaris* and *Strongyloides*. The hookworm incidence remains consistently high, as in the other Bangweulu Locations. The two cases of *S. haematobium* were an adult female and a female child of 7 years. The latter had haematuria and was heavily infected judging from the numbers of eggs that were being passed. She was said to have lived here all her life but it is difficult to reconcile this with the fact that all the other 41 children examined were negative. The adult case had come from Mpika district 3 years previously and may have contracted the infection there or elsewhere.

The only snails collected at Nsalushi were *Lanistes* and *Viviparus* which were common but not very numerous in the swampy foreshore. Both these types are widespread in the Lake and Swamp, where they are preyed upon by the rich variety of bird life which abounds there.

Location Eight. (Muchishye River.)

This small stream crosses the Nsombo-Luwingu road 15 miles north of the Lake and flows into the swampy estuary of the Lupoposhi River. The Location, which is about 300 ft. above lake level, consists of two villages near the stream and three villages about 12 miles from the lake, and was chosen as a contrast to the Nsombo Location and to find out if possible the range of the Bangweulu " immunity " to Bilharzia. Of 50 individuals examined, 54% had hookworm and 4% *Strongyloides*. No other helminth infections were found. A few *Lymnaea* were collected in the Muchishye R.

Location Nine. (Upusikilo.)

Upusikilo is still further away from the Lake being 30 miles due north of it and probably 500 ft. or more above it. Seven villages near the Lufubu River were sampled and of 37 individuals examined 32.4% had hookworm and apparently related with this very low incidence is the complete absence of *Strongyloides*. The only other helminth infections noted were *S. haematobium* (one case) and *S. mansoni* (one case). The water snails *Bulinus natalensis* (believed not to be a vector of Bilharzia), *Lymnaea* and *Lanistes* were collected in the Lufubu River.

Summary of Helminthic Infections in Luwingu District.

The principal helminth infections in this district are hookworm (67.5%) *Ascaris lumbricoides* (18.2%) and *Strongyloides* (15.6%). (See Table II.) Hookworm has its highest incidence of over 70% in the

Lake and Swamp but falls away considerably in the rising country to the north. The fall coincides with the change over from the Lake Basin agricultural system to the Chitemene system and may be correlated with this transition but various other factors must also be taken into consideration such as the change from a swampy environment to one of small rivers and semi-permanent streams, the less congested population distribution and possibly also the slightly higher altitude. *Strongyloides* and *Ascaris* also fall away in the higher country. The latter species

TABLE II.

Helminthic infections in Natives of Luwingu District.

	Adults		Children		Totals
	Male	Female	Male	Female	
Number examined for intestinal helminths	52	171	107	98	428
Hookworm	61·6%	64·3%	75·7%	67·3%	67·5%
Ascaris lumbricoides ...	9·6%	22·8%	12·1%	21·4%	18·2%
Trichuris trichiura ...	0%	1·7%	2·8%	2·0%	1·8%
Strongyloides spp. ...	15·4%	14·0%	14·0%	20·4%	15·6%
Enterobius vermicularis	0%	1·1%	1·9%	3·0%	1·6%
Schistosoma mansoni ...	0%	0%	0·9%	0%	0·2%
Unidentified trematode.	0	1 Case	0	0	0
Number examined for urinary helminths ...	52	171	106	97	426
Schistosoma haematobium	0%	0·6%	0·9%	3·0%	1·2%

reaches a high incidence (62%) in one of the swamp islands, Nsalushi, which is probably representative of this type of settlement. The different percentage rate of infection in males and females with *Ascaris* indicated in Table II, is statistically significant.

The almost complete absence of Bilharzia in Luwingu District has already been commented upon and will be discussed in a later section.

Aquatic Molluscs from Luwingu District.

Species.	*Locality.*
Bulinus natalensis (Küst).	Lufubu R., Upusikilo. (9).
Lymnaea natalensis Krs.	Muchishye R., (8).
Lanistes ovum Trosch. juv.	Lufubu R., (9) Chisi Island (6).
L. magnus Furtado	Lake Bangweulu.
Viviparus mweruensis (Smith)	Lake Bangweulu.

KAWAMBWA DISTRICT.

The last 100 miles of the Luapula River together with part of Lake Mweru forms the western boundary of this district and holds more than

half the total population of 75,000 natives in a densely congested belt along the river and lake margin. Elsewhere they are thinly and unevenly scattered except in Luena Wantipa which holds over 10,000 natives. The altitude varies from about 3,000′ in the Luapula Valley to over 4,000′ in the central and eastern part of the district. Rainfall figures are 51.35″ at Kawambwa boma while from the valley and the lake the low figures of 41.1″ and 31.41″ have been recorded. Temperature data are somewhat meagre and unreliable but according to local opinion the Luapula Valley enjoys a tropical climate and very high temperatures are experienced there especially during the latter part of the dry season.

Locations were sampled in four different topographical regions in the district; in the high country in the vicinity of Kawambwa boma; in the marshy expanses to the east and north-east of it; on the east shore of Lake Mweru; and along 50 miles of the Luapula valley. Two kinds of agriculture are involved, the Lake Basin system (cassava) in the Luapula-Mweru Locations and the Western Chitemene (cassava with supplementary millet) in the others.

The heavily populated Luapula Valley region, from Johnston Falls to Kasembe, which might well be described as a village fifty miles long and less than a mile in breadth, is sub-divided into five Locations for the purpose of illustrating a geographical trend in the incidence of certain helminth infections which otherwise might be overlooked.

Location Ten. (Johnston Falls and Kashiba.)

A few miles after its descent at Johnston Falls, the Luapula river enters, at Kashiba, the widening belt of swamps referred to earlier. The population sample examined in this Location therefore consists of people living near to the river itself.

At Mambedena Mission (Johnston Falls) 57 students were examined for urinary helminths and 84 villagers at Kashiba for urinary and intestinal helminths. The *S. haematobium* incidence at Mambedena was particularly high, 69% of those up to 15 years being infected and 33% of those over 15 years. At Kashiba the incidence was 39% and 27% respectively. The infection rates with hookworm *Strongyloides* and *S. mansoni* are indicated in Maps III, IV and VII. Many *Physopsis globosa* were collected from an inlet of the river—the "Kabula water" —a very likely source of the *S. haematobium* infection.

Location Eleven. (Nsakalubu.)

With the increasing width of the swamp downstream, the line of villages is pushed farther away from the river. At Nsakalubu three swamp margin villages were sampled and the helminth infections in 40

individuals were as follows: hookworm (55%), *Strongyloides* (32.5%), *S. haematobium* (one case) and *S. mansoni* (two cases). The sudden drop in the *S. haematobium* incidence is noteworthy and it is consistently low in the subsequent downstream swamp-margin villages. *S. mansoni* on the other hand becomes increasingly prevalent.

Location Twelve. (Lukwesa and Kapala.)

The swamp narrows from Lukwesa to Kapala and the line of villages approaches the river for a distance of 5 miles. At Kapala they diverge again and become increasingly distant from one another down to Lake Mweru. Helminthic infections in 88 individuals at Lukwesa and Kapala were as follows: hookworm (62.5%), *Strongyloides* (29.5%), *S. mansoni* (15%), and *S. haematobium* (two cases).

Location Thirteen. (Luamfwe and Lubule.)

As the swamp widens the line of villages is crossed in several places by small streams which find their way into large lagoons (Kaombe and Pemba) in the middle of the swamp. Luamfwe (41) and five villages at Lubule (36) were sampled with the following results. Hookworm (78%), *Strongyloides* (32.5%), *S. mansoni* (43.8%), and *S. haematobium* (one case). The sharp rise in the *S. mansoni* rate is largely due to Luamfwe where 66% were infected, yet a careful search in this Location failed to reveal the sources of the infection. No snails of the genus *Biomphalaria* were found in the streams or in the lagoons though *Bulinus natalensis* and *B. tropicus* were not uncommon.

Location Fourteen. (Kasembe.)

This is the most northerly swamp-margin Location and is situated near the mouth of the Ngona river which rises in the higher country a few miles west of Kawambwa town. The helminth infections here do not differ very markedly from those of the previous Location. In 79 examinations there was hookworm (75%), *Strongyloides* (21.5%), and *S. mansoni* (9%) but there were no cases of *S. haematobium*.

Location Fifteen. (Ngona River.)

Leaving the Luapula River, a population sample was examined in the higher country to the east, at the source of the Ngona River near Kawambwa town. This is a fairly thickly populated settlement and well circumscribed. In 71 examinations there was hookworm (50%), *Strongyloides* (11%), *S. mansoni* (two cases), *S. haematobium* (two cases) and *Enterobius* (one case). The small number of bilharzia cases, three of which however were in children, probably indicates that the infection is endemic here in a low degree, although no potential snail vectors could be found in the locality.

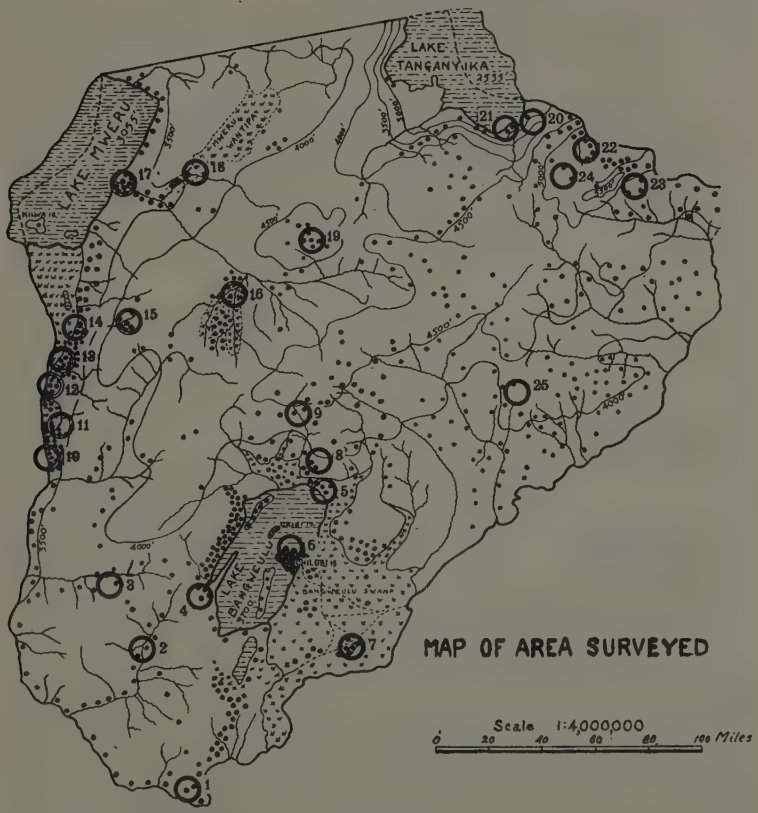
Location Sixteen. (Luena Wantipa.)

The confluence of the rivers Pambashye and Lufubu, 25 miles east of Kawambwa town, is characterized by a considerable tract of swampy country the margins of which are well populated. In 80 examinations there was hookworm (72.5%), *Strongyloides* (9%), *S. mansoni* (34%) and *S. haematobium* (two cases). Conditions resemble those of the lower Luapula Locations and with the exception of the relatively low incidence of *Strongyloides*, the helminth infections are also similar. In drainage channels from wells and springs entering the swamp the following snails were found; *Biomphalaria tetragonostoma, Segmentina planodiscus* and *Bulinus natalensis*. Both of the Planorbid species, *B. tetragonostoma* and *S. planodiscus*, were naturally infected with a non-human form of bilharzia.

Location Seventeen. (Kafulwe.)

The last few miles of the Kalungwishi River and part of Lake Mweru shore indicate the position of a settlement of some 5,000 natives where this Location was set up. The topography of the area requires but brief description. The country is undulating and falls rather steeply at the lake shore. It is sparsely wooded and poorly supplied with perennial small streams, hence the inland and riverine villages are dependent largely upon wells during the dry season. The annual rainfall is low, judging from the 2-yearly mean of 31.41" recorded at Kafulwe.

Population samples were examined from villages in the vicinity of the lake, from riverine villages and inland villages. The combined infection rates in these three localities are as follows : hookworm (61%), *Strongyloides* (32.4%), *Ascaris lumbricoides* (one case), *S. mansoni* (42%), *Hymenolepis nana* (one case). Hookworm was highest (69.2%) in the riverine villages and lowest (48.7%) in the vicinity of the lake. 28 out of 39 or 71.8% of the lakeside villages were infected with *S. mansoni*. After a short search the probable source and probable snail vectors of this infection were found in a small stream (Kafulwe?) which was being used extensively for watering and washing. This ran through a banana plantation for part of its length where shade and moisture and privacy provided ideal conditions for the transference of *S. mansoni* eggs to the stream. Large numbers of *Biomphalaria pfeifferi* were found in the stream especially in clear water at the lower end of the plantation where washing was being done. 22% of these were emitting cercariae of the human bilharzia type. Experimental verification of their identity was unfortunately not feasible at the time. In association with *B. pfeifferi* were large numbers of *Lymnaea caillaudi* vars. *undussumae* and



Map II. Showing the principal physical features of the area surveyed; the position and numbers of the Locations (black circles) and the approximate population density and distribution. (Each black dot represents 500 persons.)

Numbers and Names of Locations.

1. Kapalala.
2. Luena River.
3. Ft. Rosebery boma.
4. Mwamfuli.
5. Nsombo.
6. Chilubi Is. and Chisi Is.
7. Nsalushi.
8. Muchishye River.
9. Upusikilo.
10. Johnston Falls and Kashiba.
11. Nsakalubu.
12. Lukwesa and Kapala.
13. Luamfwe and Lubule.
14. Kasembe.
15. Ngona River.
16. Luena Wantipa.
17. Kafulwe.
18. Makupa.
19. Mporokoso boma,
20. Niamkolo.
21. Mpulungu.
22. Kawimbe.
23. Mambwe.
24. Lunzuwa.
25. Kasama.

humerosa none of which were showing any kind of infection. *Bulinus natalensis* was collected in another stream (Munwa) near the Kalungwishi River.

Summary of Helminthic infections in Kawambwa District.

The prevalent infections are hookworm (63.2%), *Strongyloides* (27%) and *S. mansoni* (23.6%). *S. haematobium* (11.8%) was found to be highly endemic only along one section of the Luapula River (Location Ten), before the latter enters the belt of swamps which persists down to Lake Mweru. Along the swamp belt and at Lake Mweru this infection

TABLE III.

Helminthic infections in Natives of Kawambwa District.

	Adults		Children		
	Male	Female	Male	Female	Totals
No. examined for intestinal helminths ...	107	211	196	144	658
Hookworm	62·6%	63·0%	64·8%	61·8%	63·2%
Ascaris lumbricoides ...	0%	0·5%	0%	0%	0·16%
Strongyloides spp. ...	24·3%	20·0%	31·1%	26·4%	27·0%
Enterobius vermicularis	0%	0·5%	0%	0·7%	0·3%
Schistosoma mansoni ...	12·1%	26·5%	17·8%	29·2%	23·6%
Hymenolepis nana ...	0%	0·5%	0%	0%	0·16%
No. examined for urinary helminths ...	124	209	223	148	704
Schistosoma haematobium	7·3%	3·8%	13·0%	13·5%	11·8%

was found to be either absent or present only on a minute degree, whilst *S. mansoni* is well established in the same region. The different infection rates with *S. mansoni* (Table III) is statistically significant in the case of males and females but not in the case of adults and children. *Ascaris lumbricoides* is notably absent, even in the Luapula swamps and Luena Wantipa, where conditions are similar, if not identical with those of the highly infected Bangweulu region.

Aquatic Molluscs from Kawambwa District.

Species.	*Locality.*
Biomphalaria pfeifferi (Krs).	Kafulwe R., (17).
B. tetragonostoma (Germain).	Luena Wantipa (16).
Segmentina planodiscus M. & P.	Luena Wantipa (16).
Lymnaea caillaudi Bgt. var. *undussumae* Mts.	Well, Luamfwe village (13).

Species.	*Locality.*
Lymnaea caillaudi Bgt. vars. *undussumae* and *humerosa* Mts.	Kafulwe R., (17).
Lymnaea natalensis Krs.	Luntomfwe R. (Trib. of Kalungwishi R.
Physopsis africana Krs.	,, ,,
P. globosa Morel.	Kabula R., Kashiba village (10).
Bulinus natalensis (Küst).	Luena Wantipa (16) Luena R. Lagoon (16).
	Luamfwe village (13) Munwa R. (17).
	Mulele R. (Trib. Luapula R.) (13).
B. natalensis (Küst), (nearest).	Swamp, Kalasa village near Kawama (12).
	Luapula R. near Kawama (12).
B. natalensis (approaching *Phys. globosa*).	Kaombe Lagoon (13).
B. natalensis and *P. globosa* (affinities with both species).	Nsakalubu R. (11).
B. tropicus Krs.	Mulele R. (13).
Cleopatra johnstoni Smith?	Luapula R. near Kawama (12).
Lanistes sp. juv.	Munwa R. (17).
Viviparus sp.	Munwa R. (17).
V. mweruensis var. *pagodiformis* Smith.	Lake Mweru (17).

MPOROKOSO DISTRICT.

Two Locations were studied in Mporokoso; one near Mweru Marsh about 30 miles from Lake Mweru and 500′ above it and the other in the still higher country to the east at the town of Mporokoso (4,570′).

Location Eighteen. (Makupa.)

Eight villages were sampled at the southern end of the marsh where it joins the Mofwe dambo. In 59 individuals examined there was hookworm (50%) *Strongyloides* (28.8%) and *S. mansoni* (5%).

This region is not thickly populated. The agricultural practice is a form of Chitemene known as the Northern Thicket which derives its name from the type of vegetation. Finger millet is thus a main crop but village garden cassava is also extensively cultivated. Perennial streams are scanty and during the dry season water holes are employed at Makupa. No snails were found in the vicinity, so that if Makupa be a

typical settlement in the marsh region it is likely that bilharzia is not endemic there.

Location Nineteen. (Mporokoso Boma.)

Two groups of villages to the north west and to the south of the town respectively were sampled. In 79 individuals there was hookworm (26.5%), *Strongyloides* (2.5%) *S. mansoni* (one case) and *Enterobius* (one case).

The low incidence of hookworm and *Strongyloides* is in sharp contrast to their occurrence in the low lying regions and climatic differences may account for this, though the agricultural practice here (Northern Chitemene) may also have some influence on it. The locality is well supplied with perennial streams of various sizes, but as no snails of any kind were found in them it can be fairly safely stated that bilharziasis is not endemic here.

TABLE IV.

Helminthic infections in Natives of Mporokoso District.

	Adults		Children		Totals
	Male	Female	Male	Female	
No. examined for intestinal helminths ...	37	30	40	31	138
Hookworm	43·2%	40%	35%	29%	37%
Strongyloides spp. ...	24·3%	16·7%	7·5%	6·4%	13·8%
Enterobius vermicularis	0%	0%	2·5%	0%	0·7%
Schistosoma mansoni ...	5·4%	6·7%	0%	0%	2·9%
No. examined for urinary helminths ...	37	30	40	31	138
Schistosoma haematobium	0%	0%	0%	0%	0%

ABERCORN DISTRICT.

The highest elevations in the plateau occur in this district, one of its peaks (Sunzu) attaining 6,782′; and also the lowest, for the southern end of Lake Tanganyika (2,535′) lies within its boundaries. Recorded rainfalls are not so high as those in the low-lying central part of the plateau. Finger millet is the staple crop, cultivated by the Northern Chitemene or Northern Grassland system, but the lake shore villages grow cassava.

Location Twenty. (Niamkolo.)

This large village on the lake shore two miles from the steamer port-of-call, Mpulungu, was sampled. In 75 individuals examined there was

hookworm (56%), *Strongyloides* (22.6%) and *S. mansoni* (61.3%). The high bilharzial incidence implies a well established local endemicity which is well suited by the local conditions of flat country irrigated by several small streams arising in the surrounding hills. Possibly on account of recent rains and flooding, no specimens of *Biomphalaria* could be found, and other species were scanty, namely *Bulinus natalensis, Bulinus forskalii* and *Lymnaea caillaudi* var. *undussumae*.

Location Twenty-one. (Mpulungu.)

In thirty individuals examined only 16% had hookworm. This low infection rate may be due partly to the fact that the population here is somewhat fluctuating and partly to the fact that at this point the pebbly foreshore, which is used extensively for personal ablutions, is unsuitable for hookworm larval development. Two cases of *S. mansoni* and two of *S. haematobium* were noted, none of which had been long resident in the locality. There were also three cases (10%) of *Strongyloides*.

Location Twenty-two. (Kawimbe.)

The next three Locations are in the highlands at altitudes of over 5,000′. In the first of these, near Kawimbe Mission, a total of 68 were examined, which include some African staff at the Mission as well as villagers. These showed hookworm (23.5%) *S. haematobium* (30.9%), *S. mansoni* (two cases) and *Taenia* sp. (one case). The source of *S. haematobium* infection was apparently the Lumi river in which *Physopsis globosa* was found, some of which were infected with cercariae of the human bilharzia type.

Location Twenty-three. (Mambwe.)

Eighty-three villagers and mission boys were examined at Mambwe Mission for intestinal helminths and 88 for *S. haematobium*. The results were, hookworm (24.1%) *Strongyloides* (one case) and *S. haematobium* (25.8%). As at Kawimbe, the hookworm incidence is low when compared with that of the lake shore Location; similarly with *Strongyloides*. *S. haematobium* is apparently extensively endemic in the Mambwe area, judging its distribution from the Mission boys who come from various outlying localities, and who were unlikely to have become infected near the Mission itself where no evidence was found of endemic *S. haematobium*. Of seven boys from villages on the Itende River about 25 miles south east of the Mission, *all* were infected. (There are some local superstitions about this river which might possibly be associated with heavy endemic bilharziasis. It is said that natives dislike the place and abandoned it for many years although it was not unsuitable for cultivation. In recent years they have been returning in

small numbers and five villages now exist. It is the local belief that people, who wash themselves in the river become ill and that if a pregnant woman bathes there she will have a premature birth. A chief will not cross the river but will make a long detour around its head waters instead; otherwise it is believed he will die.)

Location Twenty-four. (Lunzuwa.)

Villagers (31) near the Agricultural Station and students (40) were examined here. 10% of the students and 29% of the villagers had hookworm. (Some of the students recently had hookworm treatment.) Other infections were *Strongyloides* (4.2%), *Enterobius* (one case), *S. haematobium* (7%) and *S. mansoni* (one case).

TABLE V.

Helminthic infections in Natives of Abercorn District.

	Adults		Children		Totals
	Male	Female	Male	Female	
No. examined for intestinal helminths ...	119	65	126	47	357
Hookworm	30·2%	40·0%	23·8%	23·4%	28·8%
Strongyloides spp. ...	8·4%	9·2%	4·7%	6·4%	7·0%
Enterobius vermicularis	0%	1·5%	0%	0%	0·3%
Schistosoma mansoni ...	7·5%	10·4%	13·4%	29·8%	14·5%
Taenia sp.	0·8%	0%	0%	0%	0·5%
No. examined for urinary helminths ...	123	65	127	47	362
Schistosoma haematobium	6·4%	6·1%	16·6%	23·4%	12·1%

Abercorn Hospital.

Thirty in-patients at the native hospital were examined. These had hookworm (43.3%), *Strongyloides* (one case) and *S. mansoni* (one case). The *S. mansoni* case was from Niamkolo and together with two hospital records from the same place provided the clue to the discovery of the highly endemic Location Twenty.

Europeans.

Twenty-four adults and five children in Abercorn District were examined for intestinal helminths. One case of hookworm in an adult male was found, which was probably not contracted locally.

Summary of Helminthic Infections in Abercorn District.

There appears to be a relationship between helminth incidence and altitude in this district which is exemplified by the four principal types.

In the highlands, hookworm and *Strongyloides* have a low infection rate compared with that on the lake shore; so also with *S. mansoni* *S. haematobium* on the other hand appears to be widespread in the highlands though with varying degrees of intensity, while it seems to be absent at lake level.

Aquatic Molluscs from Abercorn District.

Species.	*Locality.*
Bulinus natalensis (Küst).	Lumi R., Kawimbe. (22). Small stream, Niamkolo. (20).
Physopsis globosa Morel.	Lumi R. (22).
P. africana Krs. (nearest).	Wawa R. (Trib. of Saisi R.,) (23).
Bulinus (*Pyrgophysa*) *forskalii* (Ehrn).	Niamkolo. (20).
Lymnaea caillaudi var. *undussumae* Mts.	Niamkolo. (20).
Lymnaea sp. juv.	Lumi R. (22).
Biomphalaria sp.	Mbulu R.

KASAMA DISTRICT.

Location Twenty-five. (Kasama.)

Four villages situated within 10 miles radius of Kasama town were examined: Namulundu (35), Kasama (60), Onole (62) and Musa (30); also 21 schoolboys at Malole Mission. The results are seen in Table VI. The helminthic infections and their incidences bear a close resemblance to those of Location Nineteen (Mporokoso Boma) which is

TABLE VI.

Helminthic Infections in Natives of Kasama District.

	Adults		Children		Totals
	Male	Female	Male	Female	
No. examined for intestinal helminths ...	60	64	50	34	208
Hookworm	36·6%	32·8%	46·0%	35·3%	37·5%
Strongyloides spp. ...	1·7%	1·5%	4·0%	0%	1·9%
Hymenolepis nana ...	0%	1·5%	0%	0%	0·5%
No. examined for urinary helminths ...	59	61	51	34	205
Schistosoma haematobium	0%	1·5%	0%	0%	0·5%

similar to the present Location in its altitude, rainfall, agriculture and dearth of snail fauna.

The figures in Table VI can hardly be taken as representative of the district as a whole since they are from such a limited area, and it is not unlikely that foci of bilharziasis will be found on the Chambezi River and its tributaries; moreover, judging from previous experience during the survey, the incidence of hookworm will be found to be higher in the southern and more low-lying parts of the district.

Summary of Results and Comparison with Southern Rhodesia.

The total examinations are summarised in Table VII, from which it is apparent that hookworm (52.2%) far exceeds any of the other helminths in respect to infection rate. Next in this order are *S. haematobium* (14.7%), *Strongyloides* (13.3%), *S. mansoni* (6.99%) and *A. lumbricoides* (3.7%). The infection rates with other species are so small

Table VII.

Helminth infection rates in combined six districts compared with infection rates in Southern Rhodesia.

	Adults		Children		Totals N. Rhodesia (Buckley)	Totals S. Rhodesia (Blackie*)
	Male	Female	Male	Female		
No. examined for intestinal helminths	523	826	722	504	2575	1806
Hookworm ...	47·2%	55·1%	53·2%	51·4%	52·2%	13·0%
Ascaris lumbricoides	1·3%	5·3%	2·6%	4·9%	3·7%	2·4%
Trichuris trichiura...	0%	0·4%	0·4%	0·4%	0·3%	0·2%
Strongyloides spp.	12·2%	12·1%	14·5%	14·5%	13·3%	2·5%
Enterobius vermicularis	0%	1·2%	0·5%	1·6%	0·85%	0·5%
Schistosoma mansoni	3·6%	8·5%	6·1%	9·3%	6·99%	11·6%
Hymenolepis nana	0%	0·24%	0%	0%	0·08%	0·88%
H. diminuta ...	0·%	0·%	0·15%	0%	0·04%	—
Taenia sp. ...	0·19%	0%	0%	0%	0·04%	1·77%
Ternidens deminutus	0%	0%	0%	0%	0%	3·5%
Trichostrongylus sp.	0%	2 cases	1 case	0%	0·1%	1 case
No. examined for urinary helminths	543	820	750	504	2617	1886
Schistosoma haematobium	8·6%	9·5%	21·7%	19·2%	14·7%	23·1%
S. mattheei ...	0%	0%	0%	0%	0%	0·5%

* These figures are compiled from the report by Blackie (1931) and comprise the combined results of examinations of indigenous natives (pp. 6–10) and of a mixed native community (p.13).

as to be almost negligible, with the exception of *E. vermicularis* whose real incidence is probably much greater than the small rate (0.85%) recorded.

Inasmuch as the technique of collecting and examining specimens was similar to that employed by Blackie (1931), a comparison between the incidences recorded in the present survey with those of the Southern Rhodesian survey is permissible and can be made from the figures set down in Table VII, but the conclusions to be drawn from such a comparison are necessarily limited on account of the different geographical procedures which were followed in the two surveys. Thus, the six districts of the present survey constitute a circumscribed bloc of territory whereas the areas surveyed in S. Rhodesia are in various parts of the country, some of them widely separated. There is a striking difference, however, in the hookworm incidences, as figured in Tables VII and VIII, which tends to confirm Blackie's belief that natives from

TABLE VIII.

Incidence of hookworm amongst indigenous natives in certain districts of Northern and Southern Rhodesia.

N. Rhodesia. (Buckley)	*Percentage infected*	*S. Rhodesia* (Blackie)	*Percentage infected*
Fort Rosebery	51·9%	Sebungwe	4·4%
Luwingu	67·5%	Darwin	—
Kawambwa	63·2%	Umtali	19·8%
Mporokoso	37·0%	Melsetter	9·1%
Abercorn	28·8%	Wankie	3·6%
Kasama	37·5%	Ndanga, Bikita etc.	2·5%

the more northerly parts of N. Rhodesia have a higher rate of infection than the indigenous natives of S. Rhodesia. The factors influencing the distribution of hookworm in the latter country have been ably discussed by Blackie, from which it can be inferred that the lower hookworm incidence there is due to a climate and physiography which are dissimilar to those of the N. Rhodesian area surveyed. The hookworm incidence in each survey is reflected in the figures for *Strongyloides* whose intensity usually parallels that of hookworm, in a lower degree.

The bilharzia incidence on the other hand is seen to be higher in natives of Southern Rhodesia, but it would be difficult to attempt to assess the significance of this difference as the factors governing the distribution and incidence of this infection are so numerous and complicated. It will be noted however that *S. haematobium* is the more

prevalent infection in both regions. A more enlightening method of comparison is seen in Table IX in which the percentage rates of infection in individual districts in the two regions are brought together and arranged in descending numerical order.

TABLE IX.

Incidence of S. haematobium *and* S. mansoni *amongst indigenous natives in certain districts of Northern and Southern Rhodesia.*

S. haematobium.

N. Rhodesia.	*Percentage*	*S. Rhodesia*	*Percentage*
Fort Rosebery	34·2%	Bikita, Ndanga, etc.	26·9%
Abercorn	12·1%	Darwin	21·8%
Kawambwa	11·8%	Wankie	21·7%
Luwingu	1·2%	Umtali	20·0%
Kasama	0·5%	Melsetter	15·8%
Mporokoso	—	Sebungwe	9·6%
(Average)	(14·7%)	(Average)	(18·3%)
		*(Salisbury Native Hospital)	29·7%

S. mansoni.

Kawambwa	23·6%	Melsetter	16·9%
Abercorn	14·5%	Darwin	9·2%
Fort Rosebery	3·6%	Wankie	6·6%
Mporokoso	2·9%	Umtali	3·5%
Luwingu	0·2%	Sebungwe	1·8%
Kasama	—	Bikita, Ndanga, etc.	—
(Average)	(6·99%)	(Average)	(7·54%)
		* (Salisbury Native Hospital)	17·3%

* Mixed native community.

It is clear from this table that the incidence of *S. haematobium* is more consistently high in the S. Rhodesian districts although the highest incidence recorded (34.2%) is from Fort Rosebery, in N. Rhodesia. It is also of interest that Blackie found the high rate of 58.7% in 63 natives from N. Rhodesia who were examined on the day of entry into S. Rhodesia.

With regard to *S. mansoni,* the incidence shows very uneven figures in the districts of both countries and illustrates the patchy distribution of this species. Here again the highest recorded incidence (23.6%) is from N. Rhodesia, in Kawambwa district. There is but slight difference between the average infection rates (6.99% and 7.54%) in the two countries, if the figures for Salisbury Native Hospital be excluded.

From the faunistic point of view, the only major differences exhibited in the two surveys are in the occurrence of *Ternidens deminutus, Schistosoma mattheei* and *Taenia* spp. In N. Rhodesia no eggs that could be definitely assigned to *T. deminutus* were found in the faecal specimens examined nor were any adults found after autopsy, but the possibility of its occurrence as a human infection in the Northern Province cannot be excluded since it was found after autopsy in the large intestine of a Little Grey Monkey at Mwamfuli in Fort Rosebery district, and Dr. P. Le Roux (personal communication) has found the baboon infected with this species in Isoka district. No eggs of the *S. mattheei* type were found in any of the urines examined. The scarcity of cattle and sheep in the area surveyed may possibly be related to this negative finding; it certainly accounts for the fact that only a single case of *Taenia* was encountered during the survey. The other species which are recorded from one and not from the other country are so rare that little comment can be made on the possible significance of their occurrence or non-occurrence.

Helminthic Infections in the Copperbelt.

The Copperbelt consists of the four mining townships Luanshya, Nkana, Mufilira and Nchanga which are situated in the north-eastern part of the Western Province and contain more than half the total European population of Northern Rhodesia. This important copper-producing region also holds a very large native population recruited for mine labour from various parts of the country and to a lesser extent from Belgian Congo. Each of the four mines employs native labour forces numbering up to 11,000 which are housed and fed in mine compounds and receive medical attention at well-equipped hospitals. As might be expected in a native community of such size and origin, a considerable variety of helminthic infections occurs; this is borne out by perusal of the records of the laboratories attached to the native hospitals. Of these, hookworm is of outstanding importance, for it is present in a high proportion (50%-70%) of newly-recruited labour. As such it might constitute a serious menace to the health of the community especially in the densely populated conditions obtaining in the mining areas and the favourable environment for its propagation in the underground workings. Particular attention therefore is given to the diagnosis and treatment of all cases of hookworm in new recruits and this initial

control is supported by the systems of sanitation which operate in the mine compounds and in the mine shafts. Under ground, this consists of well-constructed bucket latrines at suitably spaced intervals along the shafts. Strict supervision and efficient routine bucket disposal reduce the possibility of hookworm transmission to a minimum. Above ground, the native lines are provided with communal latrines having water-borne sewage system. Here also hookworm transmission is under control, though probably to a lesser degree than in the mine shafts since the strict observance of latrine usage is not so easily enforced and the infection may be kept going by defaecation elsewhere in the compound, especially by children. In the rainy season too, natives tend to squat near their huts instead of going to the latrines.

TABLE X.

Analyses of water samples at Nchanga.

Locality	*Copper in parts per million*	*p*H	*Snails*
Kafue R. " 22 mile landing "	0·10	7·9	*Physopsis globosa* juv., *Lymnaea caillaudi*, *Cleopatra* sp. juv. (All scarce.)
Kafue R. " Hippo Pool."	0·11	8·2	*Bulinus natalensis* juv., *Planorbis costulatus*. (Both scarce.)
Nchanga R. Golf course	0·08	6·9	No snails found.
Malemba R.	0·02	8·1	" " "
Mushishima R.	0·06	8·1	" " "
Duck Dambo	0·10	6·7	" " "

Bilharziasis is also present in native recruits but with a much lower incidence than hookworm. The sanitary measures in the compounds also act as a control for the possible spread of this infection by such individuals who might have escaped diagnosis and treatment on admission. Furthermore, the environment of the compounds is usually so much altered from the natural state by constructional work and other results of mining activities that snail habitats tend to become obliterated. Further afield, mosquito control measures, which include the canalisation of natural streams, must also function as a means of bilharzia control. That potential bilharzia vectors do occur in the Copperbelt

however, is seen from the results of a rapid but by no means exhaustive survey of water courses in that area, which are indicated in the list below.

The scarcity of snails at Nchanga, where the native ore is an oxide of copper, compared with the other mines where it is copper sulphide, led to the suggestion that the copper content in local waters at Nchanga might possibly be high enough to be an inhibiting factor to snail existence. Accordingly water samples were taken from five rivers and one dambo which had been searched for snails. Through the courtesy of Dr. E. Pinkney, Chief Chemist at Nchanga, analyses of these were obtained, the results of which are seen in Table X.

There appears to be no correlation between the presence or absence of snails and the amount of copper in the waters concerned, nor with the *p*H of these waters, but it is possible that either or both of these factors may account for the general scarcity and under-development of the snails. Mozley (1944) found that 30% to 53% of *Biomphalaria* died in 24 hours in water containing 0.1 part per million of copper and 77% to 97% died in water containing 0.2 p.p.m. He is also of the opinion that a *p*H of 7.5 or over may be a condition unfavourable for snails.

Aquatic Molluscs from the Copperbelt. (*W. Province.*)

LUANSHYA.

Species.	*Locality.*
Physopsis globosa Morel, juv.	Kafubu R.
P. globosa Morel.	Luanshya R.
Planorbis natalensis Krs. or *P. costulatus* Krs. (very worn.)	Kafubu R.
Lymnaea caillaudi Bgt.	Luanshya R.
L. caillaudi Bgt. (nearest).	Kamilienda R.
L. ? nyanzae Mts.	Balubu R.
Cleopatra ferruginea Lea (nearest).	Kafubu R.
Lanistes ovum Troschel juv.,	Luanshya R. Kafubu R.
No snails found.	Fisensa R.
,, ,, ,,	Mukomo R.

NKANA.

Species.	*Locality.*
Physopsis globosa Morel.	Small pond near Mwambeshi R.
Bulinus hemprichii depressus Haas.	Kamfinsa R. Stream running through Old's farm.
Biomphaladia pfeifferi (Krs.)	Kamfinsa R.
Planorbis natalensis Krs.	,,
Lymnaea caillaudi Bgt. var. *undussumae* Mts.	Small pond near Mwambeshi R.
L. natalensis (Krs.) (Dwarf race).	Kamfinsa R.
Cleopatra ferruginea (Lea.)	Small pond near Mwambeshi R.

MUFILIRA.

Species.	*Locality.*
Bulinus hemprichii depressus Haas.	" Sunken Pool ".
Planorbis natalensis Krs?	Mutundu R.
Lymnaea natalensis (Krs). (nearest).	" Sunken Pool ".
Lanistes ovum Trocshel juv.	Mutundu R.
No snails found.	Kansusu R.
,, ,, ,,	Kafue R.

NCHANGA.

Species.	*Locality.*
Physopsis globosa Morel, juv.	" 22 mile landing ", Kafue R.
Bulinus natalensis (Krs.) juv.	" Hippo Pool " Kafue R.
Planorbis costulatus Krs.	,, ,,
Lymnaea caillaudi Bgt. var. *undussumae* Mts.	" 22 mile landing ", Kafue R.
Cleopatra sp. juv.	,, ,,
No snails found.	Mushishima R.
,, ,, ,,	Malemba R.
,, ,, ,,	Nchanga R.
,, ,, ,,	Luango R.
,, ,, ,,	Duck Dambo

HELMINTHIC INFECTIONS IN PATIENTS AT NDOLA NATIVE HOSPITAL.

A random sample of 98 in-patients at Ndola Hospital was examined for intestinal helminths, and 47 for urinary helminths with the following results: hookworm (37.7%), *A. lumbricoides* (3%), *Strongyloides* (7%), *H. nana* (3%), *S. mansoni* (7%), *S. haematobium* (21%). The natives examined were from various districts in N. Rhodesia.

Aquatic Molluscs from Ndola District. (W. Province.)

Species.	*Locality.*
Physopsis globosa Morel (nearest).	" Swimming Pool ", Itawa R.
P. africana Krs. (near *globosa*).	Ndola R.
Bulinus natalensis (Küst) or *P. globosa* Morel	Ndola Swamp.
B. natalensis juv. (referable to *depressus* Haas).	,, ,,
Planorbis costulatus Krs.	" Swimming Pool, Itawa R.
Lymnaea caillaudi Bgt. juv.	,, ,, ,, ,, Ndola Swamp.
L. caillaudi Bgt. var. *undussumae* Mts.	Ndola Swamp.
Cleopatra sp. juv.	,, ,,
Bithynia stanleyi Smith.	" Swimming Pool ", Itawa R.
Lanistes ovum Troschel.	Ndola Swamp. Itawa R.
Plotia tuberculata (Müll.)	,, ,, ,, ,,
Cleopatra ferruginea (Lea).	Ndola Swamp.

Aquatic Molluscs from other parts of N. Rhodesia and from adjacent localities in Belgian Congo.

LUSAKA. (CENTRAL PROVINCE.)

Species.	*Locality.*
Physopsis globosa Morel.	Thomas's Farm Dam.
Bulinus natalensis (Küst.) (aff. *P. globosa.*)	Fish ponds, Chilanga.
B. natalensis (Küst.)	Kabulonga Dam.
Bulinus sp. (aff. *tropicus* Krs.).	,, ,,

LUSAKA (ctd.).

Species.	*Locality.*
Bulinus (*Pyrgophysa*) *forskalii* (Ehrn.).	Borrow pit, Broken Hill Road.
Biomphalaria pfeifferi (Krs.).	Kabulonga Dam. Ayrshire Farm Dam.
Lymnaea natalensis (Krs.).	,, ,, ,, ,,
L. natalensis (Krs.)?	Fish ponds, Chilanga.
Corbicula africana (Krs.).	Kabulonga Dam.

KAFUE. (CENTRAL PROVINCE.)

Species.	*Locality.*
Bulinus (*Pyrgophysa*) *forskalii* (Ehrn).	Kafue R.
Biomphalaria sp. indet.	,,
Planorbis natalensis Krs.	,,
Lymnaea sp. indet.	,,
Lanistes ovum Troschel.	,,
Viviparus sp. indet.	,,

SAKANIA. (BELGIAN CONGO.)

Species.	*Locality.*
Physopsis globosa Morel.	Stream entering swimming pool.
Bulinus natalensis (Krs.).	Water supply pumping station.
Biomphalaria pfeifferi (Krs.).	,, ,, ,, ,,
B. pfeifferi (Krs.).	Irrigation drains in vegetable garden, near swimming pool.
Lymnaea caillaudi Bgt. (var. *undussumae* Mts.)	Stream entering swimming pool.
L. caillaudi Bgt.	Water supply pumping station.
Plotia tuberculata (Müll).	,, ,, ,, ,,

CHIBAMBO. (BELGIAN CONGO.)

Species.	*Locality.*
Bulinus natalensis (Küst.)	Inlet from Luapula R.
Biomphalaria sp. indet.	,, ,, ,, ,,
Lymnaea caillaudi Bgt.	,, ,, ,, ,,
Cleopatra morrelli Preston?	,, ,, ,, ,,

Discussion.

In this section of the report the individual helminthic infections encountered in the survey are discussed separately from various aspects but with special reference to their incidence and distribution, which in the case of the principal helminthic infections are illustrated by a series of maps.

Hookworm.

Prevalent species of Hookworm. An attempt was made to determine the prevalent species in the country by collecting and examining adult worms after anthelmintic treatment and by autopsy. A total of 235 hookworms were thus collected from 20 positive cases at the African Hospitals at Lusaka, Ndola, Luanshya, Fort Rosebery and Kasama. All of these proved to be *Necator americanus*. The number of female worms collected was about twice that of males.

Intensity of infection in individual cases. The number of hookworms, whether in an individual case or as an estimated hookworm load in a community, is one of the main criteria by which the importance of hookworm infection is assessed. This is calculated by counting worms recovered after treatment or autopsy, or by egg-counting techniques, both of which methods are laborious and time-consuming, and to give results of value in the case of a community must necessarily be carried out with a large number of individuals. Egg-counts were not undertaken on the present survey and the number of individuals from which hookworms were recovered after treatment or autopsy is too small to be of much significance, but the details of these are recorded here.

At Lusaka, 22 Africans who had been diagnosed as hookworm-positive in the laboratory were given anthelmintic treatment and from their stools adult hookworms were subsequently recovered in the following numbers; 78, 41, 11, 9, 5, 5, 2, 1, 1, 1, 1, 1. From the remaining 10 cases, no worms were obtained, probably implying that their infections were very light.

At Ndola, 12 hookworm positive cases from the African Hospital, were treated but no hookworms were recovered from the stools. (The treatment here may have been inadequate for expelling the worms. Instead of the usual routine dosage with CCl_4 and oil of chenopodium, only CCl_4 was administered. It is reasonable to conclude however that the infections were probably light.)

At Fort Rosebery, 7, 6 and 3 hookworms were recovered from

three Africans who were treated at the hospital. From one positive case at Kasama hospital, 16 worms were recovered.

Three autopsies at Luanshya African hospital yielded respectively 33, 5 and one hookworms and at Ndola one autopsy yielded 8 hookworms.

From these findings it would appear that hookworm infestations are light in degree, since in 88% of cases the number of worms recovered was less than 10, and in only one case did it exceed 50.

Incidence and distribution of hookworm in the area surveyed. Hookworm was found to be universal in the Chambezi-Luapula region. It was present in every Location sampled, rarely with an incidence of less than 20% and frequently over 50%. The incidence varies in different geographical zones which are illustrated in Map III. Thus, Locations having incidences of 50% and over are situated mainly in the west and south-west of this region, while those with incidences of 40% or less are mainly in the north and north-east. (There are four exceptions to this rule, namely Location Twenty, 50%, in the north-east, and Locations Two, 40%, Three, 37%, and Nine, 32%, in the south-west.) The reason for this zoning is attributable to climatic differences associated with altitude or to different agricultural practices. The zone of high incidence obviously occupies the most low-lying part of the region, namely Bangweulu and the Luapula Valley, while Locations with relatively low incidences are mainly at the higher altitudes. The climatic factor here involved must be temperature since according to available records, there is no correlation between rainfall and altitude. The high incidence in Location Twenty in the north-east zone of low incidence, is explicable, on this theory, by its low altitude near Lake Tanganyika.

With regard to hookworm incidence and agriculture, there is also some correlation, for in the north-east zone the principal crop is finger-millet, grown by the Chitemene system already described, while in the south-west zone, cassava is the staple crop. As has been suggested earlier, there are good reasons for believing that the nature of the crop and the distance of the gardens from the villages may have an important influence on hookworm dissemination. Cassava gardens are usually near the villages, hence there is a tendency for defaecation places to become localised, over-used and heavily stocked with hookworm larvae. Millet gardens on the other hand are usually distant from the villages and this must result in a more scattered distribution of faecal deposits and lighter soil infestation with larvae. This is also well illustrated by the hookworm incidence in each of the six districts. Fort Rosebery,

Luwingu and Kawambwa in the S.W. zone have high incidences and grow cassava as the staple crop. Mporokoso, Abercorn and Kasama in the N.E zone have low incidences and grow finger millet. (See Table

Map III. Figures in circles represent the percentage rates of infection (to the nearest integer) with hookworm in each Location.

XI.) The exceptionally low rate in Location Nine, in Luwingu, is explicable by the fact that it is a millet growing locality, but the low incidences in Locations Two and Three, in Fort Rosebery, are not easily accounted for. The other aspect of agricultural practice, namely the shifting of villages involved in the Chitemene system, is probably of lesser importance, in view of the short life of hookworm larvae in the

soil. During the dry season hookworm infection is at a minimum, the soil tends to become sterilised of hookworm larvae and there can be no cumulative stoking-up of larvae even in the vicinity of permanent villages from one wet season to another. Shifting villages therefore gain no protection from hookworm infection as a result of their migrations.

TABLE XI.

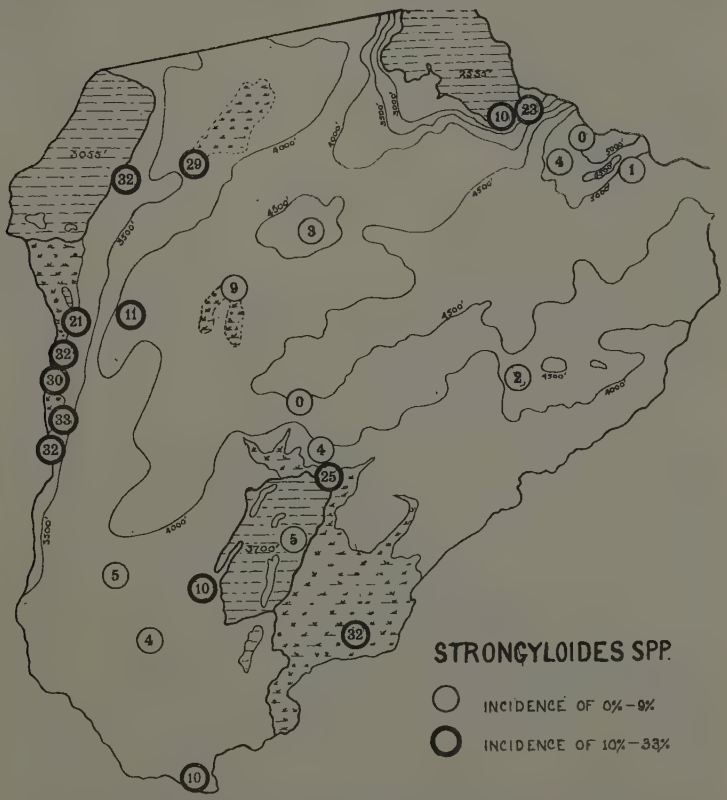
Relation of staple crop to hookworm incidence in six districts.

District	Hookworm incidence	Staple crop
Fort Rosebery	51·9%	Cassava
Luwingu	67·5%	Cassava
Kawambwa	63·2%	Cassava
Mporokoso	37·0%	Finger-millet
Abercorn	28·8%	Finger-millet
Kasama	37·5%	Finger-millet

Importance of hookworm in the area surveyed. Hookworm infection is not synonymous with hookworm disease and a high incidence in a community does not necessarily imply that individual infestations are heavy. Incidence figures unfortunately, in themselves, give little information as to the rôle played by hookworm in the general health of a community. Such data can only be acquired with certainty by means of egg counts, worm counts, blood tests and clinical observations on a large number of cases and preferably over a long period of time. Such a period should include a time of food scarcity in the native's existence, for it is then that the effects of hookworm infection are most likely to make themselves felt and hookworm disease become patent. It may well be that in famine times the ever-present hookworm in the plateau native not only exerts the devitalising effects for which it is notorious but also new infections are less easily thrown off or kept down to small numbers. It is the opinion of persons having considerable knowledge of the plateau native that while in times of plenty he is not averse to the physical exertion required for crop cultivation, in famine times his capacity for work diminishes out of all proportion and even the stimulus to produce more food is insufficient to counteract his lassitude. It is probable that hookworm is the responsible agent.

STRONGYLOIDES SPP.

The species of Strongyloides. It will be noted that this helminthic infection has been referred to hitherto in this paper only by a generic name. The reason for this is that preliminary laboratory work in Lusaka



Map IV. Figures in circles represent the percentage rates of infection (to the nearest integer) with *Strongyloides* spp. in each Location.

with faecal specimens from N. Rhodesian natives, containing *Strongyloides* larvae, showed that *S. fulleborni* was present as well as the better-known species *S. stercoralis*. Faecal specimens from six positive cases in the hospital had been cultured in the usual manner in order to

develop the free-living sexual forms from which a species diagnosis can be made; of these, four proved to be *S. fulleborni* and only two *S. stercoralis*—a proportion which was the reverse of what might have been anticipated. At Ndola hospital, cultures were made from faeces of two positive patients and these proved to be *S. fulleborni* and *S. stercoralis* respectively. It was apparent that both species occur as human infections in N. Rhodesia and that it would be erroneous to assume, on finding larvae in a faecal specimen, that these belonged to the species more commonly associated with human infection, namely *S. stercoralis*. In the field survey which followed, the investigation of the relative incidence of the two species had, for the most part, to remain in abeyance; but it was facilitated to some extent in the lower Luapula Valley by the frequency of cases and the high temperatures encountered there, which favoured the rapid development of faecal cultures in the field laboratory. Faecal specimens from six positive cases were cultured there and all of these proved to be *S. fulleborni*. At Makupa's (Location 18) three faecal specimens which were cultured, also proved to be *S. fulleborni*. The evidence, such as it is, indicates that *S. fulleborni* may be the more prevalent human infection in N. Rhodesia.

Incidence and distribution of Strongyloides *in the area surveyed.* On account of the resemblance between the external development of *Strongyloides* and hookworm, inasmuch as their larval stages feed and grow and become infective in the soil, and the similar method of invading the human host, the incidence and geographical distribution of these helminthic infections tend to parallel one another in regions where they are co-endemic. This is well illustrated in the present survey, which as in the case of hookworm, also reveals zones of high and low *Strongyloides* incidence which are similar in range with those of hookworm (see Maps III and IV). The similarity is not absolute however, for the *Strongyloides*—hookworm incidence *ratio* varies considerably from place to place. This ratio is highest in the zone of high incidence and falls appreciably in the zone of low incidence. In the zone of high incidence the ratio is most consistently high in the regions of lowest altitude, namely the lower Luapula Valley and the shore of Lake Tanganyika. From these data it may be tentatively concluded that the external development of *Strongyloides* is even more susceptible to variations in environment than is hookworm, and that the critical factor here involved is altitude, and hence presumably, temperature. This conclusion is supported by the experimental work of Cordi and Otto, 1934, and of Bruns, 1937.

ASCARIS LUMBRICOIDES.

Incidence and Distribution. Unlike hookworm, this helminthic infection was *not* found to be widespread in the area surveyed. As can be seen in Map V it is only a sporadic occurrence outside Lake Bangweulu and the adjoining swamp; in the latter regions its incidence varies with the different topography of the Locations which were sampled. Thus, in the swamp island Location (Nsalushi) it attains its maximum incidence of 62%; in lake island Locations (Chilubi and Chisi) its average incidence was much lower being 16%, while in the lake shore Locations (Mwamfuli and Nsombo), its average incidence of 7% was the lowest.

This patchy distribution is merely another example of what epidemiological studies on *A. lumbricoides* have revealed in various countries throughout the world during the past 20 years—studies which have included detailed enquiries into the reasons for the local prevalence of the infection, from every relevant aspect. The extensive literature which has accumulated on this subject will not be quoted here in full; it has been reviewed comprehensively in a recent paper by Shikobalova (1943).

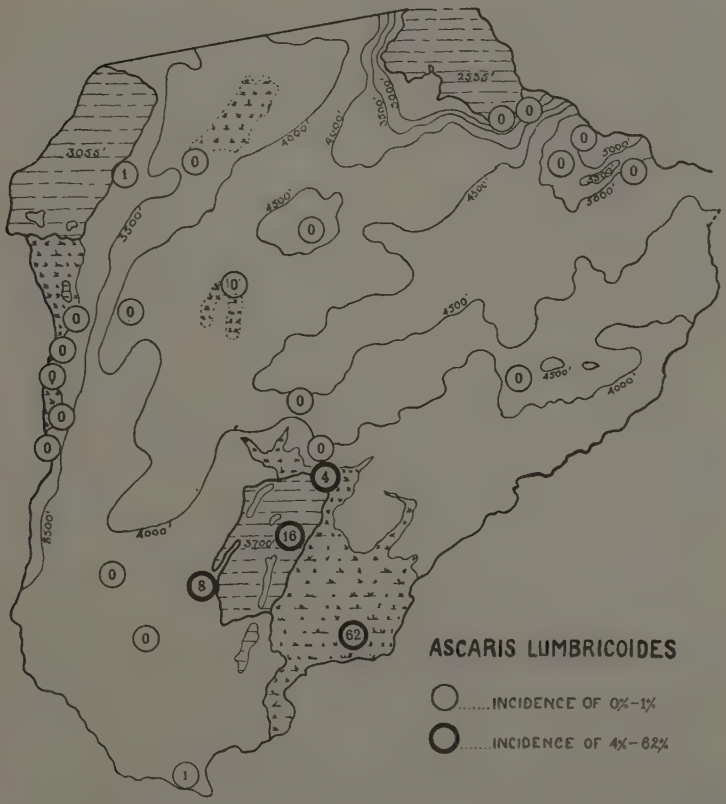
The major factors involved in any explanation of the local prevalence or scarcity of *A. lumbricoides* are, climate (including micro-climate), sanitation and personal hygiene. The subsidiary factors which derive from these are numerous, sometimes interacting and often complicated. Any attempt to account for the *A. lumbricoides* incidence in a community requires a very complete knowledge of local conditions such as temperature, precipitation, atmospheric humidity, sunlight, soil moisture, soil type, vegetation, shade, population density, child population, personal hygiene standards, defaecation habits and faeces disposal, all or many of which may be involved simultaneously with varying degrees of importance. That the task of interpreting the individual or collective influence of these factors in relation to infection incidence is a difficult one, hardly needs stating. It is well exemplified by the conclusions of Scott (1939) from the results of an epidemiological study of *Ascaris lumbricoides* in Egypt, where marked differences in incidence were found between various districts, as well as sharp local differences. Similarly, in the present survey, marked variations were encountered, but in the absence of detailed knowledge of all the factors concerned, only tentative explanations can be put forward.

Taking firstly the Bangweulu region and analysing the incidence data obtained in the three topographical areas referred to above, it is found that the incidence in the swamp island Location, *viz.* 61.9% of 84

persons examined is significantly different from either the incidence in the lake island Location (15.9% or 132 examined) or that of the lake shore Location (7.38% of 244 examined). The difference between the lake island incidence and lake shore incidence is not significant, so that the discussion resolves itself into a comparison between swamp island Location and lake Locations. If temperature, rainfall, humidity and amount of sunlight be admitted to be common factors in the two places, then a simple explanation presents itself *viz.* that the relatively high incidence in the swamp island is due to the congestion of the population into a small area which is further decreased during the seasonal flooding. This results in defaecation close to houses or not very distant from them in the muddy swamp margin which provides a good medium for the development of the eggs; the absence of overhead shade and hence of protection from lethal sunlight is counteracted by the muddiness of the water or the thick masses of vegetation on the foreshore. (In some swamp settlements dense masses of vegetation form the actual foundation of the villages.) It may be assumed that ingestion of infective eggs occurs either by drinking polluted water or by adhesion of egg-infected mud to the hands and subsequent food contamination. In the case of the less heavily infected lake Locations, congestion must also be a critical factor, especially in the islands, but the seasonal rise in water level does not limit the available area for defaecation sites to the same extent and the sub-stratum is probably not so favourable for egg development as that provided by the swamps. (The lake shore Locations, Mwamfuli and Nsombo, should on this theory show a much lower incidence than was observed. The difference between their incidence, 7.38%, and that of the lake islands, 15.9% has been shown *not* to be significant; but the fact that this is statistically a border-line case tends to favour the theory put forward.)

Considering secondly, the scarcity of the infection in the rest of the area surveyed, the problem of disclosing the inhibiting factors there, at first sight a simple one in view of the strongly contrasting incidence and topography with those of the Bangweulu area, on closer examination proves to be complicated. In the higher parts of the plateau the inhibiting factors are apparently (1) absence of population congestion, (2) staple crop and agricultural system (whose influence on hookworm incidence has already been discussed and could similarly influence *Ascaris* incidence) and (3) the low temperatures, especially in the rainy season when conditions of moisture for egg development are most favourable. But in the lower-lying, moister, warmer and more

congested places such as the Lower Luapula Valley and Luena Wantipa (Location Sixteen) the inhibiting factors are not obvious. Either the parasite, which probably exists there as a low-grade infection, has only



Map V. Figures in circles represent the percentage rates of infection (to the nearest integer) with *Ascaris lumbricoides* in each Location.

recently been introduced and has not yet had time to become firmly and widely established, or, in the words of Scott (1939) the scarcity is due to " a summation of minor factors, each of which is unrecognisable when acting alone ".

Sex and age incidence of Ascaris lumbricoides. Analysis of the sex and age incidence of *A. lumbricoides* in the Bangweulu Locations, Nsombo, Chilubi Is., Chisi Is. and Nsalushi show that females have a higher incidence than males. Of 125 males examined, 14.4% were positive and of 216 females 27.8% were positive. This difference is significant and is in the same direction in every group except Nsalushi. Of 178 adults examined, 24.7% were positive and of 163 children 20.8% were positive. In no individual group is the difference between adults and children significant; nor is the difference between adults and children significantly different in males and females. This remains true when the 8 groups are pooled.

At Mwamfuli (excluding the negative " inland " village Msaila, 6 miles from the lake shore) 119 natives were examined. These comprised 55 males (14.5% positive), 64 females (7.8% positive), 54 adults (7.4% positive) and 65 children (13.8% positive). Analysis of these figures shows no significant differences between any of the possible groups.

Trichuris trichiura.

Eight cases only of this infection were encountered in the survey, 3 of which were from Chilubi Island (3.3%) and 5 from Nsalushi (6%). Its distribution thus resembles that of *Ascaris lumbricoides* in that the cases were only found in the Bangweulu area and there was a higher incidence in the swamp island than in the lake island. A similar range of distribution of *T. trichiura* and *A. lumbricoides* is to be expected on account of resemblance between their respective life-cycles, but the incidence of the two species is not necessarily equal nor parallel in areas where they are co-endemic. Laboratory and field observations of various workers have shown that the eggs of *T. trichiura* are less resistant to the influence of external factors than are those of *A. lumbricoides,* hence the incidence and distribution of the latter species are generally greater than those of the former; except in areas with high rainfall and humidity and dense shade, where *T. trichiura* is the more prevalent species (Cort, Stoll, Riley and Sweet, 1929). The moist conditions in the Bangweulu area therefore favour the spread of *T. trichiura* while the absence of shade may be the dominant controlling factor.

Enterobius vermicularis.

Twenty-one cases of this infection were diagnosed during the survey and of these 18 were in Fort Rosebery and Luwingu Districts. Diagnosis was made solely by finding eggs in faeces or urine by the routine method which was being employed. (In 5 cases, all females,

eggs of this helminth were found in urine only.) The low incidence of 0.8% recorded in the whole area would no doubt have proved much greater had the cellophane anal swab method been used.

This infection is not uncommon amongst European children in Northern Rhodesia and its cure is generally reported as being difficult. At least, apparent cures are effected, but the reappearance of the infection in the treated individual a short time later, in spite of precautionary measures taken against self-reinfection, tends to throw doubt on the efficacy of the treatment. Recent researches however, on the biology of the eggs and the discovery that they can develop and remain viable in places other than on the human skin, which provides the optimum environment, show that re-infection may occur in spite of prevention by personal hygiene. It has been found that the eggs are very resistant to physical and chemical agents and that temperature and humidity are important influences on the length of time they can survive in the exterior. High temperatures and low humidity are quickly lethal, but low temperatures prolong their viability if there is sufficient humidity. The eggs, which are produced in large numbers, may drop from the skin of an infected person and become lodged on a variety of household objects such as bedding, floors, furniture etc., where they remain viable for periods which vary with the surrounding temperature and humidity, and can infect or re-infect the members of a household. Thus, in addition to the finger-to-mouth mode of infection, dust borne infection is possible and does take place (Cram, 1943 and Schuffner, 1944). Control by hygiene is therefore difficult and it would appear that repeated or continuous treatment, over a period long enough to outlast the viability of the eggs scattered about the house, together with the usual measures of personal prophylaxis, offer the best prospects of control.

Filarial Infections.

Blood films were examined for evidence of filariasis in Africans at the following hospitals : Lusaka (172 individuals examined), Ndola (172), Fort Rosebery (20) Santa Maria Mission (Chilubi Island) (40), Abercorn (69), Kasama (70) and Malole Mission (67).

The total number of night blood films examined was 459, and amongst these 25, or 5.4% showed *Mf. perstans*, and 3, or 0.6% were positive for *Mf. bancrofti*.

The total number of day blood films examined was 151; ten, or 6.6% of these were positive for *Mf. perstans*.

Blood films examined at Fort Rosebery, Chilubi Island and Abercorn were all negative. *Mf. perstans* was present in those examined at Lusaka, Ndola and Kasama and it is evident that this is an endemic infection in some parts of N. Rhodesia—a conclusion which merely endorses the findings recorded previously in laboratory reports from various parts of the country.

The position with regard to the endemicity of *W. bancrofti* is less certain. *Mf. bancrofti* was found, during the present survey, in the blood of three adults at Lusaka, Ndola and Kasama respectively; but none of these had been permanently resident in the country and might have become infected elsewhere. One of them was an adult male native of Tanganyika Territory, only two years resident in N. Rhodesia; the second was an adult female, native of Portuguese East Africa, four months resident in N. Rhodesia; the third was a young adult male native of N. Rhodesia, from Kashinga village, near Malole Mission, who had spent three months in Tanganyika Territory followed by two years at Lusaka. Since on this evidence most of his life had been spent in his village, it was possible that further cases might be found there. Accordingly, night blood specimens were taken from 67 individuals in villages in that locality but none were positive; neither did enquiry produce any evidence of elephantiasis in that area.

The first record of *Mf. bancrofti* in N. Rhodesia appears to have been from Mazabuka in 1938 (Medical Report on Health and Sanitary Conditions for the Year 1938), when three cases were reported by the Medical Officer. In the Medical Report for 1943, three more cases were reported, but the history and movements of the infected individuals did not rule out the possibility of their having contracted the infection in some other country. In a recent personal communication from the Health Department, Northern Rhodesia, evidence is to hand that *Mf. bancrofti* is present in natives of Feira District, near the Luangwa-Zambesi confluence; but here again there is some doubt as to whether the infections were contracted locally or during visits to adjoining countries.

Definite clinical evidence of filariasis due to *W. bancrofti* in N. Rhodesia is also lacking. The condition known as "thick leg", "Serenje leg" or "Feira leg" is a form of elephantiasis in N. Rhodesian natives which is believed to be non-filarial in origin and is said not to resemble closely the elephantiasis of *W. bancrofti.* (Medical Report on Health and Sanitary Conditions for the Year 1937.) Fisher

(1941) described cases of acute thrombophlebitis in natives in the Copperbelt and suggested that this disease is widespread in the country and one of the principal causes of " thick leg ".

While it has yet to be definitely proved that *W. bancrofti* occurs as an endemic infection in N. Rhodesia, there can be no doubt that it occurs in neighbouring countries where Rhodesian natives become infected and remain infected after returning to this country; in what numbers, is unknown, since the finding of *Mf. bancrofti* in blood films has mainly hitherto been merely incidental, during the course of searches for other parasites such as malaria or trypanosomes. The public health significance of *W. bancrofti*-infected individuals in N. Rhodesia depends, of course, on the co-existence of mosquito vectors of the parasite.

More information is desirable concerning the potential danger of the infection becoming endemic in localities from which it has been reported, if indeed it is not already endemic there, and this could best be obtained from an accurate knowledge of the distribution of the mosquito vectors in the country; but especially in suspected localities, where mosquito surveys, together with systematic filarial surveys, would clear up the question of the endemicity of *W. bancrofti*.

Concerning other human filarial infections, no positive data were acquired in the present survey, possibly for geographical reasons. Microfilariae of *Loa loa* were not found in the blood specimens examined, and random skin examinations for Onchocerciasis in natives at Lusaka hospital (71), Abercorn hospital (24) and Kasama hospital (27) were all negative.

Some evidence of the existence of *Loa loa* in N. Rhodesia is however forthcoming from Dr. B. de Meillon (personal communication) who found *Mf. loa* in the blood of six natives from N. Rhodesia, all from districts in the Southern Province, namely, Mankoya, Balovale and Senanga. The question of the endemicity of these cases awaits investigation.

Human Onchocerciasis has never been recorded in N. Rhodesia. On the present survey biting Simuliidae were observed on one occasion only, when a single specimen of *Simulium neavei*, vector of *Onchocerca volvulus* in parts of East Africa, was taken on human bait near the Kaombe River in Serenje district.

Bilharzial Infections.

Incidence and Distribution of Schistosoma haematobium. The percentage rate of infection with this parasite varies considerably in different

Locations in the area surveyed, from zero to over 60%. These percentages fall readily into two groups; those of less than 10% and those of over 20%. Locations having incidences of over 20% fall into two geographical groups, namely, Locations 1, 2, 3 and 10, to the south-west of the area, and Locations 22 and 23 to the north-east. (See Map VI.)

Incidence and Distribution of Schistosoma mansoni. The incidence of this infection also varies in different Locations, from zero to 61%, and here again two groups of high and low incidence respectively are apparent, though not so clearly demarcated as is the case of *S. haematobium*. Furthermore, the "high" incidence group, here taken as 9% and over, is geographically more scattered than that of *S. haematobium*. There is clearly a grouping of "high" incidence Locations along the Lower Luapula and Lake Mweru, but elsewhere they are isolated, namely, at Kapalala (1), Luena Wantipa (16) and Niamkolo (20). (See Map VII.) It is notable that, except at Kapalala, the Locations with high *S. mansoni* incidences never coincide with those having a high *S. haematobium* incidence.

Age and Sex Incidence of S. haematobium. It has been remarked earlier that children have a larger proportion of infections than adults. Adults (577 examined) and children (533) from Fort Rosebery, Lower Luapula, and Abercorn highlands showed incidences of 20.6% and 45.9% respectively. This difference is significant and is also significant in each area. The difference between the incidence in males (35.3%) and females (30.2%) is not significant.

Haematuria. Haematuria in association with *S. haematobium* infection was commoner in children than in adults. In 256 positive children haematuria was observed in 41.4% while in 123 positive adults it was seen in only 26%.

Age and Sex Incidence of S. mansoni. Unlike *S. haematobium*, the difference between the infection rates of adults and children with *S. mansoni* is not significant. At least, this is the case in 4 out of 5 of the endemic localities whose figures were analysed. These are as follows; Kapalala, adults 9.1%, children 8.6%; Lower Luapula, adults 15.7%, children 16.4%; Kafulwe, adults 49.2%, children 35.8%; Luena Wantipa, adults 20.7%, children 41.2%; Niamkolo, adults 43.9%, children 82.4%. Only in the case of Niamkolo is the difference significant. In the combined five localities the difference is not significant, the incidences being 20% of 510 adults examined and 25.2% of 456 children examined. With regard to sex incidence *S. mansoni* also differs from

S. haematobium, for the figures show that a larger proportion of females is infected than males. This is the case both in adults and children. The incidences are as follows : female adults 23.2%, female children 32.7% ; male adults 13.5%, male children 20.4%.

Map VI. Figures in circles represent the percentage rates of infection (to the nearest integer) with *Schistosoma haematobium* in each Location.

Microscopic examination of faecal specimens at Niamkolo, where the infection rate was highest and *S. mansoni* eggs seemed to be most numerous in the stools, revealed no signs of blood or mucous.

Snail Vectors of S. haematobium. Several attempts were made to determine the snail vectors at Lusaka by exposing snails to infection with

miracidia hatched from eggs passed by patients at the African hospital, but no conclusive results were obtained. The species which were employed were *Physopsis globosa, Bulinus natalensis* and *B. forskalii* all of which had been collected in the vicinity of Lusaka.

A number of *P. globosa* collected at Thomas's Farm dam were found to be naturally infected with cercariae of the human bilharzia type. Two guinea pigs were exposed to infection with these and both of them became heavily infected with adult schistosomes in the liver and mesenteric veins. These closely resembled *S. haematobium* but as only males were present an accurate identification could not be made.

Epidemiological evidence as to the snail vector was sought during the field survey. Snails were collected or searched for in every Location visited with the object of finding some geographical correlation between snail species and bilharzia species. The distribution of snails of the families Bulinidae and Planorbidae in the area surveyed is depicted in Maps VI and VII in which it is apparent that either *Physopsis globosa* or *P. africana* is present in every Location where *S. haematobium* is clearly endemic, namely, Locations 1, 2, 3, 10, 22 and 23, and, with the exception of Mwamfuli (Location 4) where a low incidence was found, these two potential vectors are notably absent elsewhere. *B. natalensis* has the widest distribution of the Bulinidae; experimental and epidemiological evidence obtained in this survey and by other workers elsewhere indicate that this species is not a vector of *S. haematobium* but the difficulty of distinguishing this species from *P. globosa* should be borne in mind. *B. tropicus* and *B. forskalii* were found in only three Locations and hardly merit consideration as possible vectors.

It may be concluded with some confidence that *Physopsis globosa* and/or *P. africana* are the vectors of *S. haematobium* in the area surveyed; but in view of the difficulty of determining accurately the species of some of the Bulinidae this conclusion is of less practical importance than academic interest and control measures against bilharzia vectors should not attempt to discriminate between different members of the Bulinidae group.

Snail Vectors of S. mansoni. Experimental infections to determine the vector of *S. mansoni* at Lusaka also gave inconclusive results. The species exposed to miracidia were *Biomphalaria pfeifferi* and *Lymnaea natalensis*.

Two species of *Biomphalaria* were encountered in the field survey, namely *B. pfeifferi* and *B. tetragonostoma*. The dimensions and general appearance of these snails are very similar, but the angularity on the

whorls of *B. tetragonostoma* and the roundness of those of *B. pfeifferi* renders them easy to distinguish.

It is highly probable that one of these species is the vector of *S.*

Map VII. Figures in circles represent the percentage rates of infection (to the nearest integer) with *Schistosoma mansoni* in each Location.

mansoni in this area, although their distribution and that of *S. mansoni* are not well correlated and are even conflicting in some aspects. Thus, in the seven Locations where *S. mansoni* is endemic (*i.e.* Nos. 1, 12, 13, 14, 16, 17, and 20) *B. pfeifferi* was found in only one Location, No. 17, and *B. tetragonostoma* in two, Nos. 1 and 16. Furthermore, both of

these snails were found in the Lwela River Location (No. 2) where *S. mansoni* is not endemic; but in the highly endemic Location 17, where many specimens of *B. pfeifferi* were collected, 22% of these were naturally infected with cercariae of the human bilharzia type. This positive evidence together with the fact that this species is known to be a vector elsewhere, indicate that it is the probable vector in the area surveyed. Concerning *B. tetragonostoma* as a vector, the evidence is mainly inconclusive.

Habitats of Bilharzia Snails. Snails of the Bulinidae and Planorbidae groups were collected from a variety of habitats, from vegetation, débris or mud in small streams, artificial or natural ponds, irrigation dams, large lagoons, inlets from large rivers, marshes and very rarely on the margins of large rivers. Most frequently they were collected in small streams, which though not necessarily the optimum habitat, are the principal habitat in the area surveyed and in the other parts of the country where they were collected. The numbers of times they were collected from different habitats are as follows:

In small streams *Physopsis globosa* (5), *P. africana* (5), *Bulinus natalensis* (13), *Biomphalaria pfeifferi* (5), *B. tetragonostoma* (3), *B. tropicus* (1).

In ponds: *P. globosa* (1), *B. natalensis* (1).

In irrigation dams: *P. globosa* (1), *B. natalensis* (1), *B. pfeifferi* (1), *Biomphalaria** sp.? (1).

In large lagoons: *B. natalensis* (3), *B. tetragonostoma* (1).

In inlets from large rivers: *P. globosa* (1), *B. natalensis* (2), *Biomphalaria** sp.? (1).

In margins of large rivers: *P. globosa* (1), *B. natalensis* (1), *Biomphalaria** sp.? (1).

In marshes: *B. natalensis* (2).

In small rivers: *P. africana* (2).

Thus, in a total of 52 collections, more than half were from small streams, which also yielded a greater variety of species than any of the other habitats. The species concerned were notably scarce, absent or undeveloped, in large rivers, lakes and swamps.

Geographical Distribution of Bilharzia and Related Factors. The dissimilar distribution of the two species of Bilharzia and the absence of both from the Bangweulu area has already been remarked upon. The latter phenomenon is undoubtedly related to the scarcity and

* Specimens lost

apparent absence of snails of either the Bulinid or Planorbid group. Large bodies of water such as lakes or swamps appear to be unfavourable habitats for these forms, which, belonging as they do to the Pulmonata Order and breathing by means of a lung and breathing tube, must come to the surface occasionally and may find the relatively deep water of lakes and swamps entirely unsuitable. Gill breathing forms such as *Lanistes* and *Viviparus* on the other hand are common enough in the lake and swamp. This explanation of the scarcity of Bulinidae and Planorbidae (also Lymnaeidae) is not however, entirely satisfactory, for the shallow water lake island margins and the dense vegetation of the swamp should provide suitable conditions for surface-breathing forms. In the absence of any positive data regarding chemical qualities of the water which might be inhibiting factors to snail existence, there still remains the more obvious explanation that natural enemies may be acting as a biological control, for the Bangweulu swamps are the breeding ground or haunt of many kinds of birds which are known to feed upon snails. I am much indebted to Mr. W. V. Brelsford, lately District Commissioner of Luwingu District, for his assistance in compiling from various sources, including his own wide knowledge of Bangweulu bird-life, the following list of birds and notes thereon, which may be important in this connection.

Snail-Eating Birds of Bangweulu.

African Open-bill Stork.

Native names: Nshibu, Fritota nkola, Mushongolankonto; all meaning " snail breaker ".

" Essentially a marsh bird frequenting shallow water in quiet bays and backwaters. Favourite food the snail Ampullaria (*Lanistes?*). Never goes far from water as it lives largely on fresh-water molluscs. Nests in the swamps and is a permanent resident in Bangweulu. It is perhaps the most numerous species there. The largest flocks, of several hundred birds together, are in the southern swamps in July and August. They roost at night in the Itili plains south of the swamp, covering acres of ground in one large black mass. They nest in the rainy season in the tall reed areas."

Hammerkop Stork.

" Lives near muddy pools and feeds on molluscs. Only seen in the southern swamps as a rule. Has been seen at Nsombo and Chilubi but is unusual in deep water."

Spoonbill.

" Feeds on crustacea, mollusca and aquatic life. Not common in Bangweulu swamps."

Flamingo.

" Sea grasses, mollusca and crustacea are the principal diet. Migratory. Visits Bangweulu swamps just at the beginning and end of the rains."

Whistling Teal.

" Feeds principally on crustacea, and molluscs. Big flocks occur on Lake Chali (south-east Bangweulu) all the year round."

Maccoa Duck.

" Feeds chiefly on water snails and water beetles. Very rare in N. Rhodesia."

Purple Heron.

" Diet not restricted to fish, but also frogs, insects, crustacea etc. Extremely common throughout the swamp area."

Little Grebe.

" Feeds on fish, larvae, crabs and water snails. Often caught in fishing nets. Widespread distribution."

White-faced Duck.

" Feeds on molluscs, crustacea, weeds and grass seeds. Seen on upper Luapula and Lake Chali.

Pin-tail Duck.

" Feeds on leaves and seeds of aquatic plants mainly, but also on crustacea and molluscs. Recorded at Ndola. (Will probably be found at Bangweulu.)"

Cape Teal.

" Feeds on seeds, leaves, insects and mollusca. Distribution. Nyasaland and S. Rhodesia. Migratory."

Garganey.

" Feeds on molluscs. Doubtful resident of N. Rhodesia."

Black River Duck.

" Feeds mainly on vegetation but also on larvae, crustacea and mollusca. Northern Rhodesia."

Yellow-bill Duck.

" Commonest species in Bangweulu. Feeds on vegetation, seeds, aquatic insects, molluscs, crustacea, frogs and tadpoles."

Pochard.

" Very common in the Bangweulu area. Next to the Yellow-bill, this is quite one of the commonest. Feeds on larvae, crustacea and aquatic molluscs."

Little Egret.

" Feeds on frogs, fish, small crustacea and mollusca."

Squacco Heron.

" Eats molluscs. Widespread in rivers, swamps and channels in the Bangweulu area."

Green-back Heron.

" Eats snails. Only seen in Luapula River."

Saddle-bill Stork.

" Eats everything that lives in water. Common in Bangweulu Swamp."

Night Heron.

" Feeds at night on aquatic life including molluscs. In lower Luposhoshi estuary in the north and in the upper Luapula and south swamps by Mbo island. Only in papyrus in the swamps and in tree shade on the rivers."

It is conceivable that the relatively thin-shelled Bulinidae, Planorbidae and Lymnaeidae may be not only more palatable to certain kinds of snail-feeding birds, than are the heavier built *Lanistes* and *Viviparus* but that their habitat being usually near the surface of the water makes them easier to find and more vulnerable to their predators.

Concerning the dissimilar distribution of *S. haematobium* and *S. mansoni* in the rest of the area, only tentative explanations are again adducible. The fact that *S. mansoni* occurs principally in the low-lying parts of the area suggests that the bionomics of the snail vector are the critical influence, and that the high temperature and shallow sluggish moving waters are factors of importance in the snails' environment.

Although the snails collected during the survey throw little light on the problem of the distribution of *S. mansoni* it must be remembered that *Biomphalaria* (the probable vector) has a patchy distribution and requires very careful and exhaustive searching in order to establish beyond doubt its presence or absence in any locality—a fact which tends to emphasise its sensitivity to environment. (Planorbids are usually found in shallow water near to the surface, and if disturbed, sink to the bottom. They crawl up vegetation to get near the surface again. Deep

water, especially if water movement causes repeated disturbance, is therefore unfavourable. The shape of the shell is also ill-adapted to running water.)

S. haematobium on the other hand, exists in the higher, and at times much colder regions to the north-east in Abercorn district, as well as in the warmer south-western part of the area, especially in Fort Rosebery district. The inference here is that the snail vector is less exacting than that of *S. mansoni* as to its environment. The rather sudden " fade-away " of *S. haematobium,* however, in the lower Luapula Valley, after the river has dropped from Johnston Falls and then enters the widening belt of swamp which persists down to Lake Mweru, is extremely interesting from the point of view of snail ecology. In the change in topography of the river, which coincides so remarkably with the sudden fall in *S. haematobium* incidence, lies the probable explanation, though a detailed analysis of the causes cannot be furnished here. The point of interest which emerges, is that only in Location Ten is there an authentic record of *P. globosa* (presumptive vector of *S. haematobium*) and that this Location has a high incidence (43%) with *S. haematobium.* (These snails were very numerous in a wide inlet or backwater of the Luapula River, from 1 to 4 feet in depth with a muddy bottom from which was growing water weed—*Ottelia* (?)—on which the snails were collected.) In the subsequent downstream Locations, Eleven to Fourteen, which are situated on the swamp margin and have low *S. haematobium* incidences, no authentic record of *P. globosa* was made, but *Bulinus natalensis* was found on 5 occasions, *B. tropicus* once and *B. natalensis* (aff. *P. globosa*) twice. This co-distribution of *S. haematobium* and *P. globosa* thus furthers the argument that this species of snail is the vector, but raises the question as to why it was not found associated with its close relative *B. natalensis* in the lower reaches. The absence of *S. haematobium* and *P. globosa* from the swampy Luena Wantipa, where *B. natalensis* was abundant, also seems significant. These data argue on the one hand a specialised relationship between *S. haematobium* and its vector and on the other, a specialised adaptation of the vector to its environment. In this connection, the suggestion advanced elsewhere, that *S. haematobium* is spreading downstream along the Luapula cannot be accepted unreservedly. The converse of this theory, namely that the infection has been *retreating* upstream with the silting up and swamp formation on the lower reaches, is equally, if not more acceptable. One is even tempted to theorise in another direction and to suggest that *S. mansoni* is a coming menace

in this region; that it has been advancing upstream from Lake Mweru's shores where it has been long established, and that with the changing topography of the Lower Luapula and ultimately the silting up of Lake Mweru itself it is destined to become a disease of considerable magnitude and importance. According to van den Berghe (1934) *S. mansoni* is the autochthonous and dominant species in the Belgian territory—Katanga—to the west and north of north-east Rhodesia, but he considers that *S. haematobium* is spreading into Katanga from the south from Rhodesia, for it has become established at Elizabethville, at several points along the Katanga side of the Luapula River and at Kilwa on the southern shore of Lake Mweru. (It was also reported in 20% of 122 natives on Kilwa Island, L. Mweru, in a Tour Report to the Health Department, Northern Rhodesia, in 1939.) On the other hand, *S. mansoni* had not at that time been reported from the Katanga side of the Luapula and van den Berghe concluded that it must be scarce in Northern Rhodesia. This conclusion is refuted by present knowledge of *S. mansoni* distribution in the latter country and it would appear that an interesting interchange of bilharzial infections may be taking place between Katanga and N.E. Rhodesia. The " invasion " of Congo territory by *S. haematobium* from the south, and from the east across the Luapula River has perhaps been reciprocated by the entry of *S. mansoni* into N.E. Rhodesia from the north *via* Lake Mweru.

The significance of age incidence in Bilharzial infections. There is a trend of opinion in recent literature on schistosomiasis to the effect that in endemic areas where gross manifestations of the disease are not apparent, it may nevertheless exist in a sub-clinical form of great insidiousness. The accurate diagnosis of such infections by laboratory methods, serological or microscopical, is obviously of great importance. The present discussion is concerned with the validity of the latter method and arises from the fact that in an infected community the diagnosis of schistosomiasis—particularly of *S. haematobium* infections—by demonstrating eggs in the excreta, reveals a higher proportion of infections in children than in adults. (Dixon, 1934, Ramsay, 1934 and the present survey). This phenomenon, designated by Ramsay as the " inverse relationship of the age of the host and the excretion of ova," has not yet been satisfactorily accounted for; but until such time as the true explanation shall be forthcoming, the validity of microscopical diagnosis of the infection in adults is open to doubt. Several possible explanations are available: 1. Acquired partial immunity. 2. Reduced opportunities of becoming infected, owing to habit, in adults. 3. Reduced egg-laying

capacity of the parasites caused by over-population in the host. 4. Host tissue reaction to the parasites, preventing easy passage of eggs into the lumen of the intestine or bladder.

1. Brumpt (1936) has set down the evidence of acquired partial immunity in schistosomiasis and strongly favours it as an explanation of the phenomenon in question. If it be the only one, then it can be assumed that the diagnosis of schistosomiasis in adults by the microscopical method is valid. In other words, the lower proportion of adults who are passing eggs is directly related to the number of adults who have acquired some immunity to the infection.

2. This theory implies that adults who became infected early in life tend to lose the infection later and in the absence of re-infection remain free of it. In this instance the microscopical method of diagnosis also gives a true representation of the incidence of the infection.

3. Ramsay (1934) put forward this theory as an explanation, in part at least, of the phenomenon. In the light of this theory, a negative diagnosis would not be wholly genuine, since, although the pathological effects concerned with egg-production are reduced, the living adult parasites may still remain a source of disease.

4. This theory has few positive data to substantiate it. It is put foward here merely as an alternative explanation which is worth consideration and study; since, even if it should prove to be only partly concerned in the present problem, it would be of considerable significance in the matter of diagnosis. The theory originates from facts observed in the present survey and which are supplemented by Ramsay's (1934) observations, namely, that in the case of *S. mansoni* there is either no inverse ratio of egg-excretion to host age or else it exists in a much lower degree than with *S. haematobium*. The difference in the nature of the organs involved might here be the determining factor; for it is conceivable that the fibrosis of the intestinal wall might offer less resistance to the passage of the eggs than would the same reaction in the *thicker* wall of the bladder. (A study of pathological material of both species from subjects of different age-groups should reveal valuable information in this direction.)

While it is admitted that this is but a brief and inadequate discussion of a complex problem it is desired to emphasize that there are some grounds for believing that a negative diagnosis by the microscopical method, at least of *S. haematobium*, does not necessarily imply freedom from the infection, and that this method should not be relied upon

entirely but should be supplemented by serological tests, especially in the case of adult individuals, should there be any suspicion or history of bilharzia infection.

Rare Helminthic Infections and Pseudo-Infections.

Trichostrongylus sp. Eggs of this nematode were found on three occasions in stools of natives of Fort Rosebery District.

Rhabditis sp. This normally free-living nematode was recorded in stools four times in Kawambwa District and once in Abercorn District. It may be a soil nematode which accidentally contaminated the faecal specimen. Pseudo-infection?

Heterodera marioni. Eggs of this plant parasitic nematode were found four times in stools of natives of Fort Rosebery District. Pseudo-infection?

Spiruridae. Eggs of this group of nematodes, possibly of *Physaloptera* sp. were found three times in stools in Fort Rosebery and twice in Kawambwa District.

Amphistomes. Viable eggs of this trematode group were found once in Fort Rosebery District. (See description in section Nsombo, Location Five.) In this connection it may be mentioned that *Watsonius watsoni,* a rare amphistome parasite of man, was recovered *post-mortem* from a Grey Monkey at Mwamfuli, Location Four.

Schistosoma spindale. Degenerate eggs, probably of this species, were recorded once in the stool of a native on Nsalushi Island. Pseudo-infection. (This species was found *post-mortem* as a heavy infection in two lechwe (*Onotragus smithemani*) which were shot near the island.)

Fasciola hepatica. Eggs of this species were found in stools of nine natives of Kawambwa and once in a European at Abercorn. Probably pseudo-infections resulting from ingestion of infected livers of goats or sheep.

Unidentified Trematode eggs. Operculate Trematode eggs of two different species were found in stools of two Fort Rosebery natives, only one record of each species. Pseudo-infection?

Hymenolepis nana and *H. diminuta.* Rare human parasites in the area surveyed. Recorded only twice and once respectively.

Taenia sp. Also a very rare occurrence, recorded only once during the survey, from eggs in the stool of an Abercorn native. Probably *T. saginata.*

Unidentified Hymenolepis sp. For a further description of this form see the section on Fort Rosebery Boma, Location Three. The three envelopes possessed by these eggs suggest that they are from a species normally parasitic in birds *e.g., H. lanceolata* from the duck. Pseudo-infection?

Conclusion.

The present survey of helminthic infections in a circumscribed region in Northern Rhodesia reveals the major occurrence of five kinds of infections and a number of others of lesser and varying importance. Hookworm infection, intestinal and vesicular Schistosomiasis, Strongyloidiasis and Ascariasis are present in the natives in percentage rates of infection which exhibit a wide range and with this is associated a geographical zoning of each kind of infection in areas or foci of high and low incidence, more or less well demarcated, and, except in the case of hookworm and Strongyloides, having a dissimilar geographical distribution. It is this great variation in intensity within the region that renders it difficult if not impossible to estimate to what extent the results of the survey reflect the incidence of helminthic infections in the country as a whole. But at least it can be predicted with some confidence that comparable surveys in other parts of Northern Rhodesia would reveal local inequalities of incidence of a similar nature. Herein lies the principal recommendation for surveys of this kind. The knowledge that a helminth infection is highly endemic in a certain area and scarce or absent in another, should prove of value not only to medical and administrative officers in charge of districts which are measured in thousands of square miles, but also as a stimulus to the initiation of local control measures, with better prospects of success than would be held out in the contemplation of large-scale campaigns against infections of uniformly high incidence. This is especially true of the bilharzia infections which show marked regional or focal zoning, thus implying a high degree of specialisation to environment and hence of vulnerability to external agencies.

Summary.

1. In the Chambezi-Luapula area of the Northern Province of Northern Rhodesia, 2,575 Africans were examined for intestinal helminths and 2,617 for urinary helminths. The examinations were carried out *in situ* in the native villages in widespread localities representing different topographical parts of the area.

2. The average infection rates of the helminthic infections found were as follows: hookworm, 52.2%; *Strongyloides*, 13.3%; *Ascaris lumbricoides*, 3.7%; *Enterobius vermicularis*, 0.85%; *Trichuris trichiura*, 0.3%; *Trichostrongylus* sp., 0.1%; *Schistosoma haematobium*, 14.7%; *S. mansoni*, 6.99%; *Hymenolepis nana*, 0.09%; *H. diminuta*, 0.04%; *Taenia* sp., 0.04%.

3. The survey revealed regions or foci of relatively high and relatively low incidence in the case of each of the major species. The relationship of this to topographical and other factors is discussed.

4. Examination of 459 Africans for *Mf. bancrofti* revealed three positives, but none of these had definitely contracted the infection in N. Rhodesia. *Mf. perstans* was found in 5.4% of those examined; this infection is endemic in the country.

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Domorganus macronephriticus n.g.,n.sp., a new cylindrolaimid free-living soil nematode.

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Introduction.

The nematodes described and figured in the following pages are specimens mounted in glycerine which were obtained in 1931 (5 males and 4 females) and in 1932 (one female) from pasture soil at Winches Farm, St. Albans. Unfortunately they were not closely examined in the living condition and it is therefore impossible to give any information about their appearance and movements when alive. The nine specimens obtained at the earlier date were passed on to the writer by Dr. D. O. Morgan (who at that time was on the staff of this Institute) and had been found by him whilst examining earthworms in connection with investigations on " gapes " in chickens and young starlings upon which he was engaged at that time, *i.e.* March, 1931. These worms, after being killed and fixed in weak formalin and processed through dilute glycerine, were finally mounted in glycerine but no detailed study of them was made until recently when, in the course of re-arranging the slide preparations of free-living nematodes, made during the course of several years, they called for identification. The single female worm obtained in April, 1932, was found on a slide along with specimens of *Anguillulina agricola, Anguillulina dubia* and a female of *Teratocephalus terrestris,* all of which had been obtained in a Baermann funnel extraction of a piece of turf taken from one of the meadows at Winches Farm.

The worms found by Dr. Morgan were, in the absence of any precise information as to their exact location when collected, considered as having been found in the course of the dissection of earthworms and were provisionally labelled as possibly coming from earthworm body-cavity. The morphological characters, however, which they revealed on close examination, coupled with the fact that a worm having the same characters was found amongst others known to occur in association with grass roots, made it impossible to retain this view and the writer has come to the conclusion that they were not from earthworms but from soil. They present certain morphological and structural features which make it impossible to identify them with any previously described

soil or fresh water nematode. As a consequence of this a new genus is erected for their reception which is named in compliment to Dr. D. O. Morgan. The specific epithet proposed is based on the fact that they possess a large excretory cell. A description of the worms is first given followed by a discussion on their structural peculiarities and systematic relationships.

Morphology.

Dimensions :*Female,* 0.625mm. to 0.98mm., a = 19.6-23 ; b = 7.5-9.5 ; c = 6.85-7.35 ; V = 48%-49.5%. *Male,* 0.62mm. to 0.76mm., a = 22.1-25.4 ; b = 7.85-9.4 ; c = 6.3-6.95.

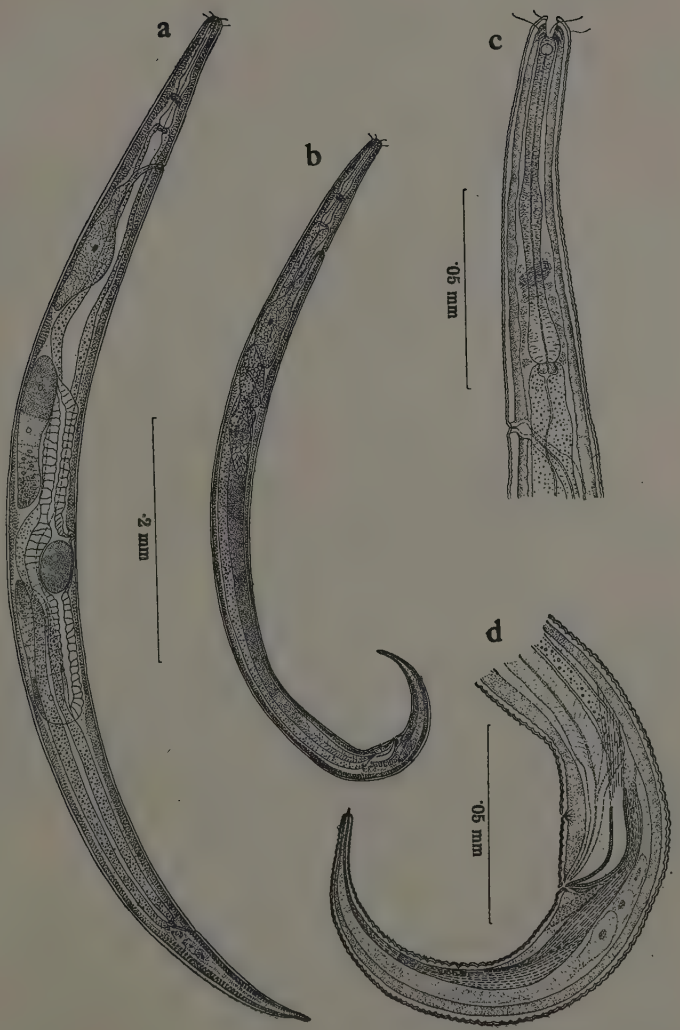
Body tapering a little in front and considerably behind to the pointed tail tip. Cuticle fitting rather loosely and provided with moderately coarse plain transverse striae. Body-wall fairly stout. Lateral fields present but un-winged, having a breadth of 1/5 to 1/4 the body width. Amphid aperture circular, leading to a cup-like amphid cavity, situated at about one head width from the anterior end. Head somewhat rounded in shape and a little flattened in front; distinct lips and papillae indistinguishable. Four delicate setae present, each about as long as head width and arising at the widest part of the head. Stoma small, beaker-shaped, about 6-7μ deep, walls thin and delicate, narrower at base than in front and deeper than wide, supported by thickened buttresses of the body-wall and leading below into the oesophagus. No oesophageal tissue surrounding stoma and no denticles or other tooth-like structures visible within it. Oesophagus in first half of its length of uniform width, cylindrical, then swelling out a little to a median corpus of somewhat fusiform shape followed by a narrow isthmus, having about the same width as the first part of the organ, and finally ending in a small but distinct, valveless bulb. The lining of the lumen is delicate throughout and the walls of the oesophagus do not appear to be very muscular. The terminal bulb probably carries glandular tissue within it. The oesophago-intestinal valve cells are comparatively small and flattened and they jut into the intestine for a short distance. The intestinal walls

Domorganus macronephriticus n.g., n.sp.

a & b.—Female and male respectively in side view, showing general shape and structure.

c.—Anterior region in side view, highly magnified, to show head and stomatal structures, shape of oesophagus and position of excretory pore.

d.—Male tail in side view, highly magnified.


a
b
c
d
·2 mm
·05 mm
·05 mm

which are comparatively stout have a fine granular appearance but call for no special description. The nerve ring lies obliquely across the isthmus of the oesophagus and numerous ganglion cells occur in its vicinity. The excretory pore occurs at a short distance behind the level of the end of the oesophagus. It lies in a shallow depression of the cuticle on the ventral body surface. The excretory duct leads inwards to a small ampulla and this is connected inwardly to the tissues of a single large excretory cell possessing a distinct nucleus and finely granular protoplasm. As shown in figs. a and b the excretory cell is a prominent feature of both male and female worms in which it overlies part of the anterior region of the intestine. The tail in both sexes is provided with caudal glands, probably three in number, which communicate with the exterior through a fine spinneret at the narrow tail tip.

Female. The vulva is inconspicuous and is slightly pre-equatorial in position. The gonads are paired, opposed and reflexed, the tip of each ovary reaching almost to the level of the vulva. The uterine walls are cellular and comparatively stout.

Male. Testes paired, short, the anterior testis reflexed but in one specimen it appeared to be outstretched. Vas deferens long, granular in structure, ending in a stouter walled region which tapers to an ejaculatory duct opening into the rectum to form a common cloacal duct. Spicules paired, ventrally arcuate, simple in structure and tapering to a point, each about 30μ long. Gubernaculum small, somewhat keel-shaped, about 8μ long. A stout band of muscles backs the spicules and is continued as a tapering strand of tissue which blends with the body wall of the ventral side of the tail. The head of each spicule is attached to a band of muscle inserted in the dorsal body wall. Caudal papillae are absent but there is a single papillate pre-anal, mid-ventral supplement situated a short distance anterior to the cloacal opening. The tail in all 5 of the male specimens was markedly flexed ventrally.

Systematics.

Absence of lateral caudal papillae (phasmids) and the presence of caudal glands opening at a spinneret at once place the new genus in the sub-class Aphasmidia of Chitwood & Chitwood (1933). In some features of its anatomy it resembles the genus *Cylindrolaimus* de Man, 1880 fairly closely but in others it differs distinctly from it. The points of resemblance to *Cylindrolaimus* are as follows: (i) absence of distinct papillate lips, (ii) presence of 4 cephalic setae and circular amphids

situated on the head, (iii) tail of the same general shape and provided with caudal glands, (iv) the presence of a single pre-anal mid-ventral supplement in the male as in *Cylindrolaimus melancholicus* de Man. It differs from *Cylindrolaimus* in the following features : (i) in the shape of the stoma which is small and beaker-like whereas in *Cylindrolaimus* it is tubular with straight rod-like walls, (ii) the cephalic setae are much longer than in *Cylindrolaimus*, (iii) in the shape of the oesophagus which has distinct median and terminal swellings whereas in *Cylindrolaimus* the oesophagus is practically of uniform width throughout with only a faint suggestion of a terminal swelling, (iv) in possessing a large excretory gland cell opening at an excretory pore situated behind the level of the oesophagus whereas in species of *Cylindrolaimus* no such gland has been observed and only in *C. obtusus* Cobb, 1916 has an excretory pore been located where it is said to be in the region of the pharynx, (v) in the ovaries being paired and opposed whereas in *Cylindrolaimus* species the female gonad is usually single and posterior though de Man (1884) suggests that in the case of *C. communis* it may perhaps be double.

The differences are such as to warrant the creation of a new genus which is considered as coming within the sub-family Cylindrolaiminae as defined by Chitwood & Chitwood (1937) if amended to include forms in which the stoma may be small and beaker-like and the oesophagus not plain but with median and terminal swellings.

Genus *Domorganus* n.g.

Diagnosis : Cylindrolaiminae emend. Small nematodes less than 1mm. long. Cuticle with plain transverse striae ; amphids circular ; head with 4 cephalic setae ; stoma small, beaker-shaped with delicate walls ; oesophagus with median fusiform corpus and a small but distinct valveless terminal bulb ; excretory pore behind level of oesophagus and leading to large excretory cell overlying fore part of intestine ; ovaries paired, opposed, reflexed ; testes paired, small ; spicules paired, simple, arcuate ; gubernaculum small, caudal papillae absent, a single, pre-anal mid-ventral papillate supplement present ; caudal glands and spinneret present in both sexes.

Type species : *Domorganus macronephriticus* n.sp.

Occurrence : Meadow soil at Winches Farm, Hatfield Road, St. Albans, England.

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Index to Volume XXI

Index of Authors

New Names in Volume XXI

New Sub-Family

New Genera

New Species

New Combinations.

INDEX—Vols. 1—21

AUTHORS

NEW ORDERS

NEW FAMILIES

NEW SUB-FAMILIES

NEW GENERA

NEW SUB-GENERA

NEW SPECIES